Diastereoselective synthesis of peptide mimetics

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DIASTEREOSELECTIVE SYNTHESIS OF PEPTIDE MIMETICS

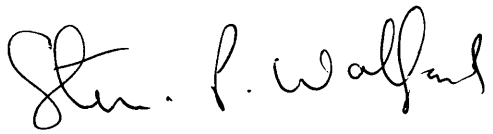
Submitted by

Steven Paul Walford

for the degree of Ph.D.

of the University of Bath

1992



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To my parents for their constant

love and support.

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I am grateful to Professor Malcolm M Campbell for his help and support. I am indebted to my industrial supervisor, Dr Martyn Pritchard, for his interest and helpful discussions throughout my stay in Cambridge. My appreciation goes to the technical staff at the University of Bath for their invaluable services. Special Thanks go to Mr John Bradley and Mr Russel Barlow (Organic Stores), Mr Dave Wood and Mr Harry Hartnell (NMR Spectroscopy), Mr Alan Carver (Elemental Analysis), Mr Chris Cryer (Mass Spectroscopy), Mrs Jo Curtis and also to June and Freda. I would also like to thank Dr Mary Mahon and Dr Kieran Malloy for the X-ray crystal structures.

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To all of you, cheers and all the best for the future.

ABSTRACT

A diastereoselective synthesis of 2,3-methanophenylalanine methyl ester afforded both the (Z)-(2S,3S)-form (**253**) and (E)-(2S,3R)-form (**259**), as major diastereomers. Each of these were incorporated, in turn, into a dipeptide analogue of CCK-4, (**260**) and (**262**) respectively. The (E)-dipeptide ester (**262**) was found to possess an excellent binding affinity for the CCK-B receptor site, IC_{50}= 6.5nM. A (Z)-dehydrophenylalanine dipeptide analogue of CCK-4 (**219Z**) was also prepared.

Conformational studies on the (Z)-dehydrophenylalanine dipeptide ester (**219Z**), and (E)- and (Z)-2,3-methanophenylalanine dipeptide esters (**262**) and (**260**), indicated the presence of a β-turn in which there was no preference in type. The phenyl and indole rings, however, did prefer to be situated on the same side of the β-turn, and thus give the molecule a suitable conformation for receptor binding.

ABBREVIATIONS

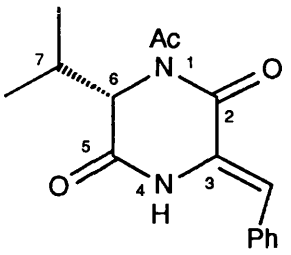
Abu	-	α-aminobutyric acid
Ac	-	acetyl
ACC	-	1-amino-1-cyclopropanecarboxylic acid
Ad	-	adamantyl
Adoc	-	adamantyloxycarbonyl
Aib	-	aminoisobutyric acid
Ala	-	alanine
Ar	-	aryl
Arg	-	arginine
Asp	-	aspartate
Azy	-	azoxy
Bk	-	bradykinin
BOC	-	*tert*-butoxycarbonyl
Bz	-	benzyl
CCK	-	cholecystokinin
CD	-	circular dichroism
C.I.	-	chemical ionisation
CNS	-	central nervous system
2D COSY	-	two dimensional correlated spectroscopy
DAST	-	diethylaminosulphur trifluoride
DBU	-	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	-	dicyclohexylcarbodiimide
DCM	-	dichloromethane
DIBAH	-	di-*iso*-butylaluminium hydride
DMAP	-	4-dimethylaminopyridine
DMSO	-	dimethyl sulphoxide

DOPA	-	3,4-dihydroxyphenylalanine
EDC	-	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EDU	-	1-(3-dimethylaminopropyl)-3-ethyl urea
E.I.	-	electron ionisation
Enz	-	enzyme
Et	-	ethyl
FAB	-	Fast Atom Bombardment
Glu	-	glutamine
Gly	-	glycine
GMP	-	guanosine monophosphate
h	-	hour
IC_{50}	-	half maximal inhibition
Ile	-	isoleucine
i-pr	-	*iso*-propyl
J	-	coupling constant
K_i	-	binding affinity
Leu	-	leucine
L-W	-	long wave
Me	-	methyl
Mes	-	mesyl
Met	-	methionine
Ms	-	2-mesitylenesulfonyl chloride
NBS	-	N-bromosuccinimide
NMM	-	N-methyl morpholine
NMR	-	nuclear magnetic resonance
n.O.e.	-	nuclear Overhauser effect
Ph	-	phenyl

Phe	-	phenylalanine
Phth	-	phthalamido
ppm	-	parts per million
Pro	-	proline
R_f	-	retention factor
RT	-	room temperature
Ser	-	serine
t-	-	*tert-*
TFA	-	trifluoroacetic acid
THF	-	tetrahydrofuran
Thr	-	threonine
tlc	-	thin layer chromotography
TMS	-	trimethylsilyl
Trp	-	tryptophan
Tyr	-	tyrosine
U.V.	-	ultra violet
Val	-	valine

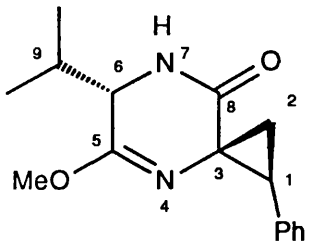
NOMENCLATURE

Examples of the nomenclature and numbering system used throughout this thesis are illustrated below, designated by an asterix, together with examples of alternative nomenclature encountered in the literature.



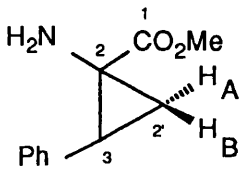
(Z)-(6S)-N(1)-acetyl-3-benzylidene-6-*iso*-propyl-piperazin-2,5-dione *

(Z)-(6S)-N(1)-acetyl-3-benzylidene-6-*iso*-propyl-2,5-diketopiperazine



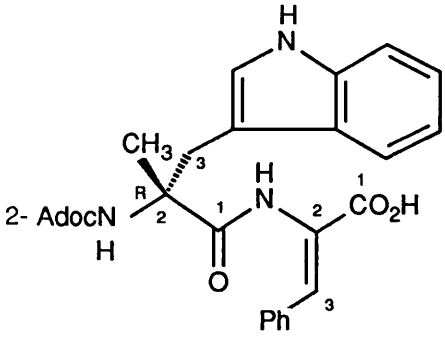
(1S, 3S, 6S)-7(H)-6-*iso*-propyl-5-methoxy-1-phenyl-4,7-diazaspiro[2,5]oct-4-en-8-one *

(1S, 3S, 6S)-7(H)-4,5-dehydro-6-*iso*-propyl-5-methoxy-1-phenyl-4,7-diazaspiro[2,5]oct-4-an-8-one



2,3-methanophenylalanine methyl ester *

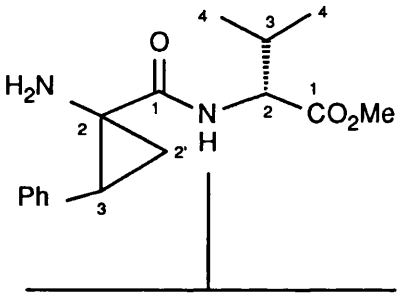
2-phenyl-1-amino-1-cyclopropanecarboxylic acid methyl ester



N^{α}(2-adamantyloxycarbonyl)-α-Me-R-tryptophanyl-(Z)-dehydrophenylalanine

In the case of the dipeptides made, the locants are numbered by treating each individual amino acid in turn, thus applying the amino acid numbering convention (IUPAC). The residue number, which is obtained by numbering the residues from the N-terminus, is placed after the atom number and separated by a full stop. For example, in Structure I, the H attached to C-3 of the valine residue in 2,3-methanophenylalanine-S-valine methyl ester is defined as 3-H.2, *i.e.* H on C-3 of residue 2.

Structure I



Residue 1 Residue 2

CONTENTS

CHAPTER 1

INTRODUCTION

INTRODUCTION

1.1 Background

Over the last twenty years there has been increased interest in the preparation of 2,3-methanoamino acids and their incorporation into various sized peptides for conformational and biological purposes. Meanwhile, a peptide mimetic of cholecystokinin (CCK), a gut hormonal regulator and neurotransmitter, was still being sought, possessing similar binding properties to CCK..

The aim of the project described in this thesis was to establish a diastereoselective synthesis of 2,3-methanophenylalanine, and to incorporate each diastereomer into a dipeptide analogue of CCK-4. The analogous dehydrophenylalanine dipeptides could then be prepared, and their binding affinities compared. These dipeptides would then be modelled in an attempt to determine their preferred conformations and thus, as a result of binding data, probe the CCK receptor requirements.

1.2 Peptide Conformation

In a peptide it is generally found that each amide bond in the backbone adopts a *trans* configuration [1]. Thus, to a first approximation, only two angles are required to define the backbone configuraton of the peptide chain: namely ϕ, which defines the torsional angle about the N^{α}-C^{α} bond and ψ, which defines the angle about the C^{α}-C (carbonyl) bond. The side chain can be characterised by the torsional angle about the C^{α}-C^{β} bond, χ.

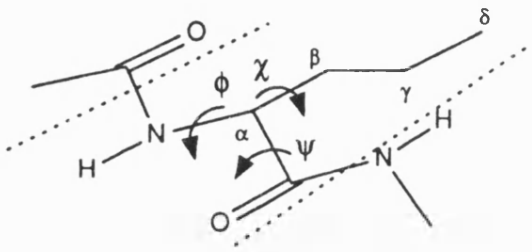


Fig. 1 A Schematic Representation Of A Peptide With Conformational Parameters Defined .

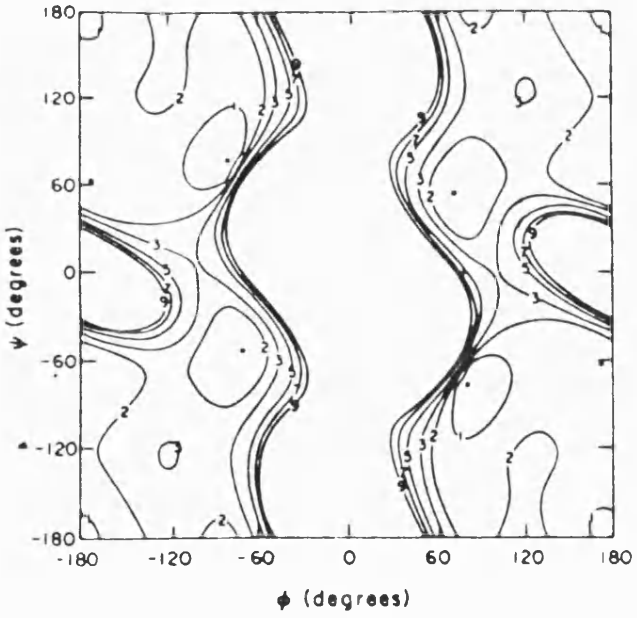


Fig. 2 Conformational energy contour map of N-acetyl-Gly-N'-methylamide

The conformational constraints on the overall configuration of a peptide can thus be usefully expressed in terms of configuration (ϕ,ψ) versus potential energy maps. These maps were first introduced by Ramachandran in 1963 [**2**] and are called Ramachandran, or ϕ,ψ, maps. Figure 2 illustrates the calculated conformational energy versus ϕ,ψ map for N-acetylglycine-N′-methylamide [**3**].

Both β-turns and γ-turns are well known secondary structures in peptides and proteins, and cause a reversal of a peptide chain over a sequence of four and three residues, respectively. These turns may or may not be stabilised by an *intra*-turn hydrogen bond. In the case of the β-turn it may occur between the carbonyl of residue 1 and the NH of residue 4, whilst in γ-turns it may occur between the carbonyl of residue 1 and the NH of residue 3. Figure 3 shows a schematic representation of both a β- and γ-turn, with the conformational parameters defined.

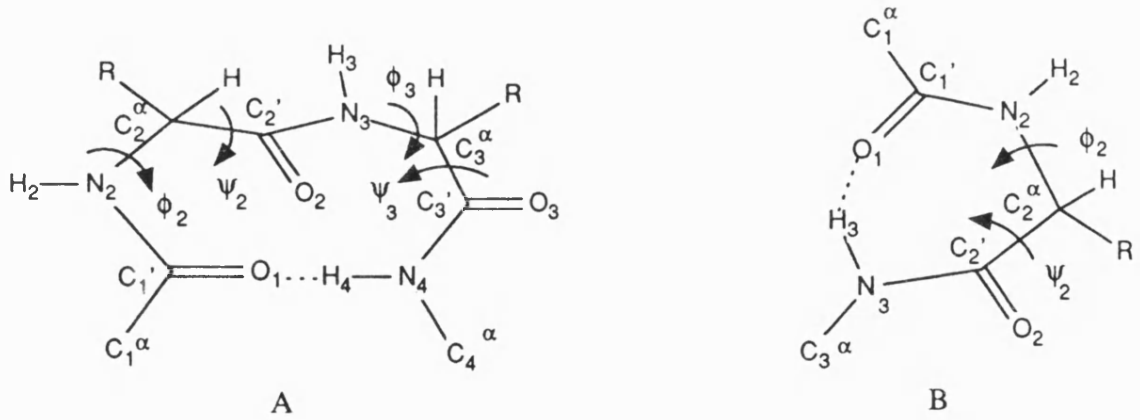


Fig.3 A Schematic Representation of (A) a β-Turn , And (B) a γ- Turn.

Conformationally, a β-turn is fully defined by the (ϕ,ψ) torsions of the middle two residues: (ϕ_2, ψ_2) and (ϕ_3, ψ_3); whereas for the γ-turn only the single torsion (ϕ_2, ψ_2) is necessary for definition. The formal definitions of types I-III β-turns and their mirror images, and normal and inverse γ-turns are given in Table 1.

Table 1 Characteristic torsions of type I-III and I'-III' β-turns [4] and γ and inverse γ-turns [5]

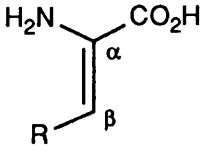
Turn	Φ, Ψ values in (°) at position 2 and 3			
	Φ_2	Ψ_2	Φ_3	Ψ_3
β-turns				
Type I	-60	-30	-90	0
Type II	-60	+120	+80	0
Type III	-60	-30	-60	-30
Type I'	+60	+30	+90	0
Type II'	+60	-120	-80	0
Type III'	+60	+30	+60	+30
γ-turns				
Turn	+70 to +85	-60 to -70		
Inverse turn	-70 to -85	+60 to +70		

It should be noted that type I and III β-turns are very similar; they occupy contiguous regions of ϕ, ψ space and are not distinct types [6]. However, it is convenient to distinguish type III as a separate category since it describes a helical turn - actually one loop of a 3_{10} helix - and may form part of a larger repeating structure.

1.3 α,β-Dehydroamino Acids and Peptides

Definition and Nomenclature

Dehydroamino acids are defined as those α-amino acids containing one or more double bonds not involved in an aromatic nucleus such as a benzene or imidazole ring. Most these are α,β- or β,γ-unsaturated amino acids (**1,2**) that occur naturally and/or have some interesting biological activity.



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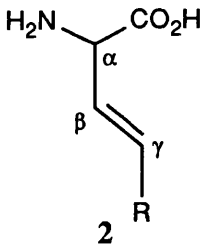

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The following is concerned only with the α,β-unsaturated variety. The nomenclature used is as follows: a capital delta (Δ) is prefixed to the three letter symbol for each amino acid residue to designate the presence of a double bond in the carbon chain, *e.g.* ΔLeu = dehydroleucine. Both structural and stereoisomers are possible in dehydroamino acids. A superscript α,β,γ, *etc.* is therefore used to designate the first carbon atom of the double bond when proceeding from the carboxyl group down the chain, *e.g.* Δ^{β}Leu indicates a dehydroleucine residue with the double bond between the β- and γ- carbon atoms. However, in the case of α,β-unsaturated amino acids this position designation is unnecessary since only the α-position is available for the double bond. Should the unsaturation appear between the C^{α} and the amino nitrogen, a superscript N will be used e.g., Δ^{N}Ala is an imine, whereas ΔAla is an enamine.

The configuration of the unsaturated site can be conveniently designated by a superscript E or Z [7] following the position descriptor, *e.g.* $\Delta^{\alpha,Z}$Leu = (Z)-α-dehydroleucine; Δ^{E}Phe =(E)-dehydrophenylalanine. In general, the (Z)-α-dehydroamino acids will have the larger β-substituents *cis* to the amino group and the (E)-compounds will have that group *cis* to the carboxyl function.

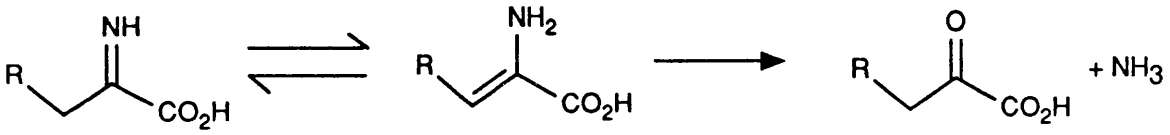
History

The chemistry of α,β-dehydroamino acids was studied extensively during the 1930's, notably by Bergmann and co-workers [8] and by Greenstein [9]. For a long time after that they received little attention, until the early 1960's when various investigations [10-12] reported their synthesis *via* non-enzymatic β-fragmentation, involving cysteine or serine residues in peptides and proteins. During the past decade, numerous α,β-dehydropeptides, many of them possessing biological activity, have been isolated from natural sources and characterized. This rapid progress was as a result of improved isolation techniques and the development of new methods of analysis. Chemical synthesis of dehydropeptides has also become possible through the recent development of new methods. These peptides have aroused interest among researchers throughout the field of peptide chemistry.

Naturally Occurring Dehydroamino Acids and Dipeptides

Free α,β-unsaturated dehydroamino acids (**1**) are unknown in nature since they are enamines and consequently might be expected to undergo ready hydrolysis, liberating ammonia and the corresponding α-keto acids (**3**) (Scheme 1).

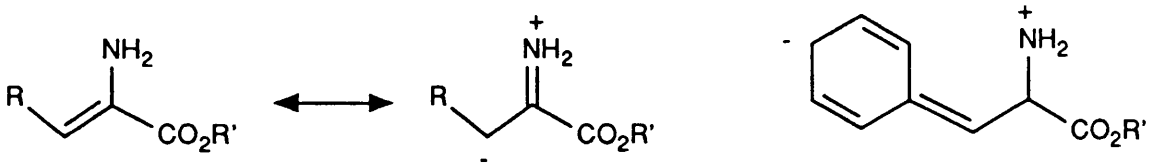
Scheme 1



4 **1** **3**

It might be expected, also, that **1** will be weakly basic and exist in tautomeric equilibrium with the imine (**4**). The weak basicity and low nucleophilicity of **1** may be ascribed to resonance stabilization of the system, in which the non-bonded electrons of the nitrogen atom are delocalized into the carbon chain. The extent of such delocalization will be enhanced when R is aromatic, as in structure C (Scheme 2).

Scheme 2



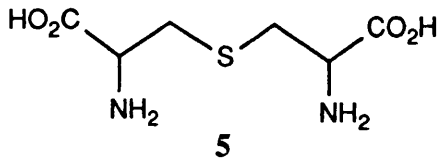
A **B** **C**

Consequently, aromatic dehydroamino acids might be expected to have amino groups showing especially low reactivity towards electrophiles, *i.e.* activated acids used in the coupling reaction of peptide synthesis. The dehydro compounds (**1**) occur in nature primarily as N-acyl derivatives or in peptide sequences, and they will be discussed in terms of these derivatives.

The majority of α,β-dehydroamino acids have been found in relatively low molecular weight peptides from microbial sources [13-15]. It was postulated that dehydroamino acids might be precursors in the biosynthesis of several heterocyclic metabolites *e.g.* penicillin and cephalosporin, which possess a variety of biological activities (Table 2).

The possibility that the tautomeric imine form (**4**) of **1** might be a key intermediate in the biosynthesis of D-amino acids from the L-isomers was also discussed [13-15]. More recently this subject has been reviewed and it was concluded that further evidence is required before it can be claimed that dehydroamino acids are necessary intermediates in the biosynthesis of either the heterocyclic metabolites or D-amino acids[16,17].

Probably the best known natural occurrence of dehydroalanine and dehydrobutyrine are seen in the microbial metabolites nisin, subtilin, cinnamycin and duramycin [18,19]. The first two peptides, nisin and subtilin, contain the two dehydroamino acids mentioned, along with "masked" dehydro-compounds in the form of lanthionine (**5**) and β-methyllanthionine (**6**). The last two peptides, cinnamycin and duramycin, contain only **5** and **6** .



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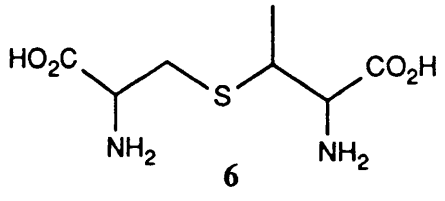

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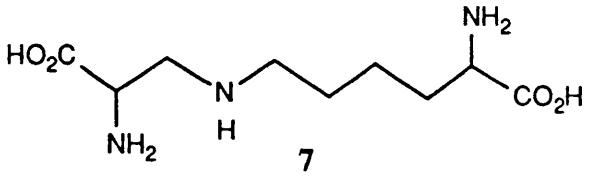

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Table 2 Natural peptides containing α,β-dehydroamino acids[a]

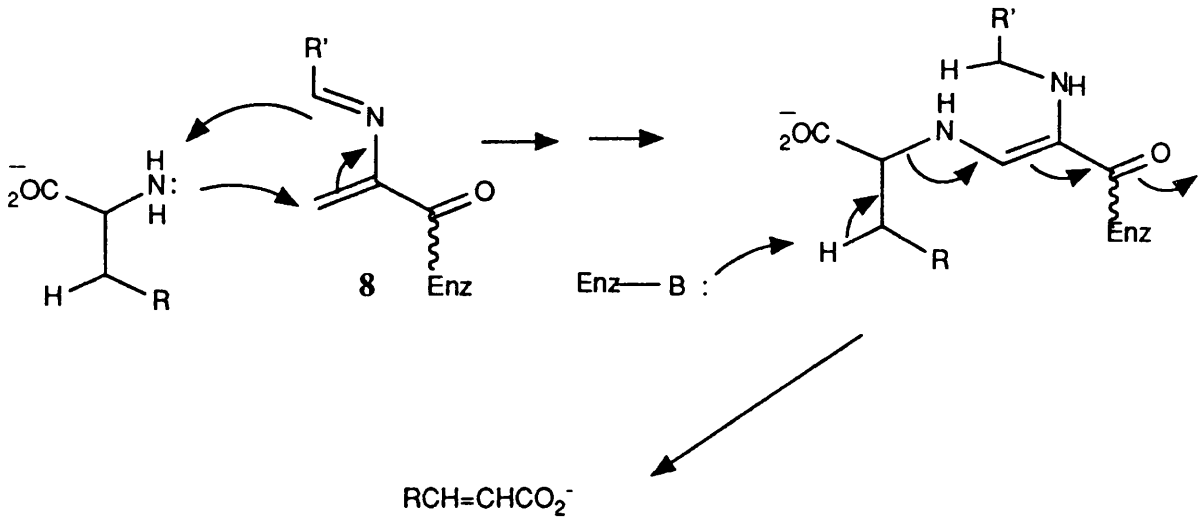
Peptide	N°. of amino acid residues	Δ amino acid involved	Biological activity	References	
				Structure	Synthesis
Amino acids					
Asparenomycin A,B and C	1	ΔPro deriv.	Antibacterial	142	
Primocarcin	1	ΔAla deriv.	Antitumour	143	144
Thienamycin	1	ΔPro deriv.	Antibacterial	145	
Piperazin-2,5-dione					
Albonoursin	2	ΔLeu, ΔPhe	Antitumour	146	147
Isoechinulin A	2	ΔTrp deriv.	Growth inhibitor	33	
Isoechinulin B and C	2	ΔAla, ΔTrp deriv.		33	
Neochinulin	2	ΔTrp deriv.		26	
Neochinulin A,D and E	2	ΔTrp deriv.		27, 31, 32	156
Neochinulin B and C	2	ΔAla, ΔTrp deriv.		29, 30	
Oligopeptides					
Celenamides A and B	3	ΔPhe deriv.	Alkaloid	46	
Cephalosporin C	3	ΔVal deriv.	Antibacterial	148	149
AM-Toxin I	4	ΔAla	Toxic	150	151
Tentoxin	4	ΔMePhe	Toxic	152	120
Lavendomycin	6	ΔAbu	Antibacterial	41	
Cirratiomycin	7	ΔIle		39	
Antrimycin	7	ΔIle	Antibacterial	36	
Polypeptides					
Stendomycin	14	ΔAbu	Antibacterial	153	
Subtilin	32	2ΔAla, ΔAbu	Antibacterial	154	
Nisin	34	2ΔAla, ΔAbu	Antibacterial	155	
L-Histidine ammonia Lyase	>100	ΔAla deriv.	Enzyme	22	
L-Phenylalanine ammonia Lyase	>100	ΔAla deriv.	Enzyme	21	

[a] ΔAbu, α,β-dehydro-α-aminobutyric acid; ΔAla, α,β-dehydroalanine; ΔIle, α,β-dehydroisoleucine; ΔLeu, α,β-dehydroleucine; ΔMePhe, α,β-dehydro-N-methyl-phenylalanine; ΔPhe, α,β-dehydrophenylalanine, ΔPro, α,β-dehydroproline; ΔVal, α,β-dehydrovaline; ΔTrp, α,β-dehydrotryptophan.

The formation of these compounds can be envisioned by an addition of the thiol group of cysteine to the electrophilic β-carbon atom of dehydroalanine or dehydrobutyrine. A third masked dehydroalanine appeared as lysinoalanine (**7**) in which the ϵ-amino group of lysine has apparently added similarly to the β-carbon of the unsaturated amino acid. The former mode leads to the complex amino acids and rings appearing in the metabolites discussed; whilst the latter mode gives a cross-linked system that may be responsible for various physical and chemical changes occurring during the heating or ageing of proteins [20].

Both of the enzymes phenylalanine ammonia lyase [21] and histidine ammonia lyase [22,23] have been shown to contain reducible electrophilic centres that appear to be at the active site. These are thought to contain dehydroalanine residues. In both cases the electrophilicity of the dehydroalanine β-carbon atom is thought to be enhanced by the existence of this residue as a Schiff base (**8**) in the enzyme, causing the amino group of the substrate to add rapidly to this site (Scheme 3).

Scheme 3



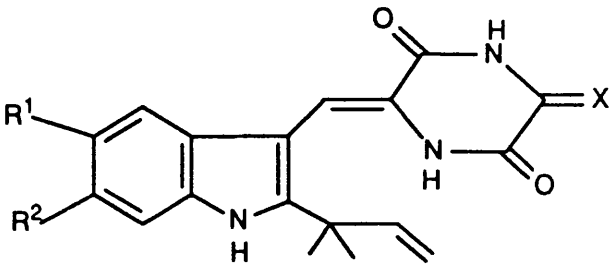
Once the amino acid is covalently bound to the enzyme, elimination of ammonia occurs to give the unsaturated product (**9**). Thus, the presence of a dehydroamino acid residue incorporates an electrophilic site into the system and leads to the Michael addition of a nucleophile to the unsaturated system. It is well known that α-acylamino acrylic acids react rapidly with thiols and amines [24] and may serve as traps for these functional groups in natural systems.

The presence of α,β-unsaturated acids is, however, not necessarily restricted to peptides of microbial origin. Dehydroalanine, for instance, has been identified in histidine ammonia lyase of both bacterial [22,23] and mammalian [25] origin and also in phenylalanine lyase from a plant source [21].

Structure and activity of α,β-*dehydropeptides*

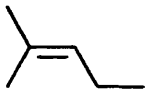
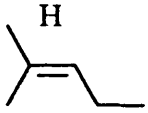
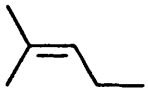
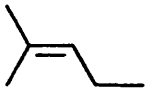
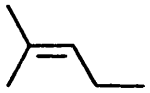
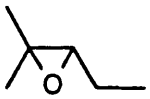
The discovery of a variety of biological activities has facilitated the isolation and characterisation of α,β-dehydropeptides from various sources. Some of these that are of particular interest because of their potential pharmacological and physiological usefulness are described here.

(a)*Neoechinulins*.- Neoechinulin is a microbial metabolite which has been isolated from *Aspergillus amstelodami* and identified as a cyclic compound (**10a**) containing a dehydrotryptophan (ΔTrp) unit with isoprenyl groups on the indole ring [26]. A number of analagous piperazindiones such as neoechinulin A (**10b**) [27,28], B (**10c**), C (**10d**) [29,30], D (**10e**) [31] and E (**10f**) [32], have been isolated and characterized in recent years. Nagasawa *et al* [33], in 1976, also reported the isolation of isoechinulin A (**10g**), B (**10h**), and C (**10i**), in which the isoprenyl group is linked to the indole ring at position 5 (R′) (Table 3).



10 a - i

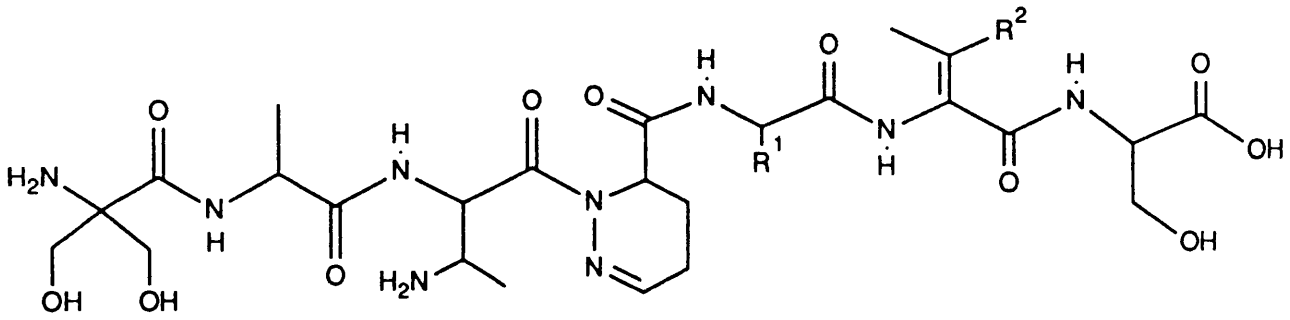
Table 3

	R^1	R^2	X
Neoechinulin (10a)	H		O
Neoechinulin A (10b)	H	H	H,CH_3
Neoechinulin B (10c)	H	H	CH_2
Neoechinulin C (10d)	H		CH_2
Neoechinulin D (10e)	H		O
Neoechinulin E (10f)	H	H	O
Isoechinulin A (10g)		H	H,CH_3
Isoechinulin B (10h)		H	CH_2
Isoechinulin C (10i)		H	CH_2

The geometry of the α,β-double bond of ΔTrp was determined to be of (Z)-type (*i.e.* the indole ring is *cis* to the amide nitrogen) by ^{1}H-NMR study [26] and ^{13}C-NMR studies [34].

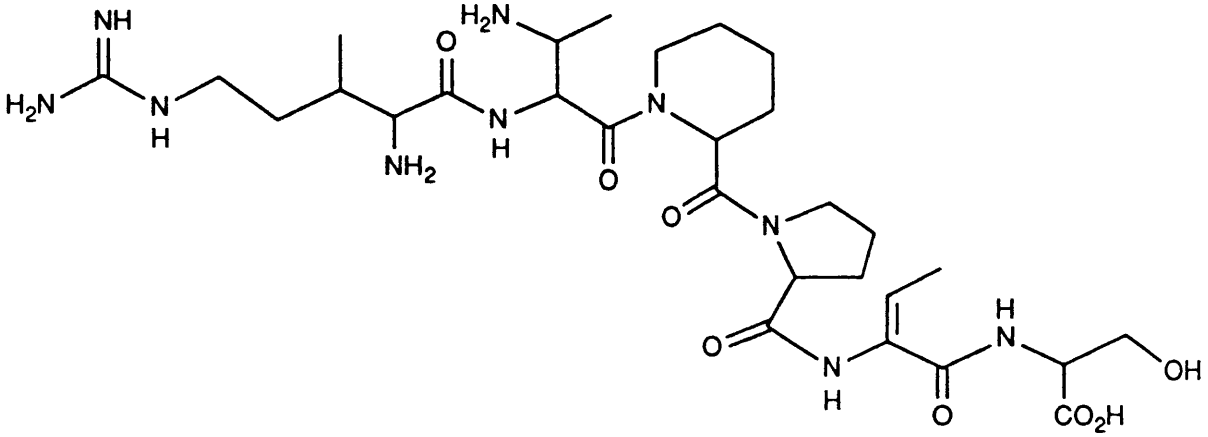
(b)*Antrimycins, cirratiomycins and lavendomycin.*- These are all fungal metabolites. The antrimycins (**11**) and cirratiomycins (**12**) are closely related and have been isolated from *Streptomyces xanthocidicus* [35-37] and *Streptomyces cirratus* [38-40]

respectively. Their structures have been elucidated and have been found to be similar. These tuberculostatic peptides contain several non-ribosomal amino acids including the previously unknown β,β'-dihydroxy-α-aminoisobutyric acid in addition to a dehydroamino acid.



11 R^1= CH_3, C_2H_5, *n*-C_3H_7, *i*-C_4H_9,
R^2= CH_3, C_2H_5,

12 R^1= CH_3, *i*-C_4H_9
R^2= C_2H_5

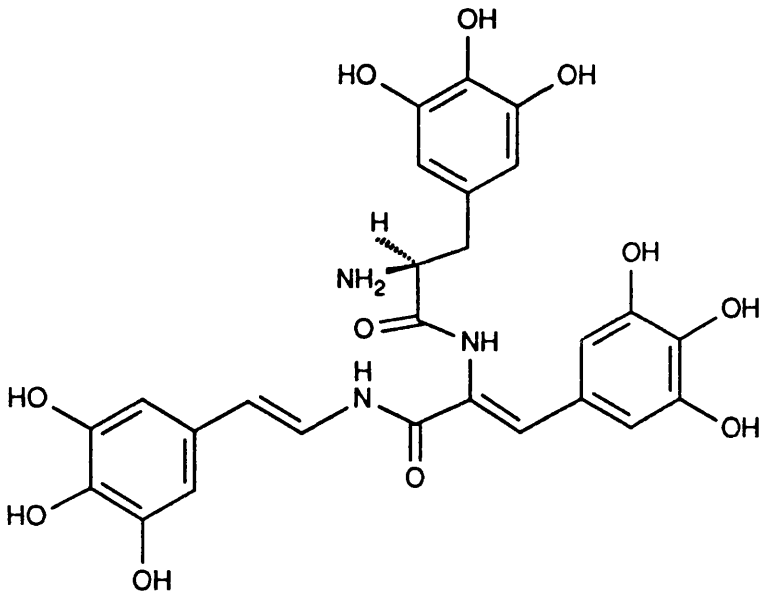


Lavendomycin (13)

Lavendomycin (**13**) is also a fungal metabolite and was obtained from the culture filtrates of *Streptomyces lavendulae* [41]. It is active against gram-positive bacteria *in vivo* and *in vitro* while having an extremely low toxicity (LD_{50}>2g/kg). This antibiotic

contains various unusual amino acids, namely dehydroaminobutyric acid(ΔAbu), α-aminocrotonic acid and the strongly basic δ-guanidinoisoleucine.

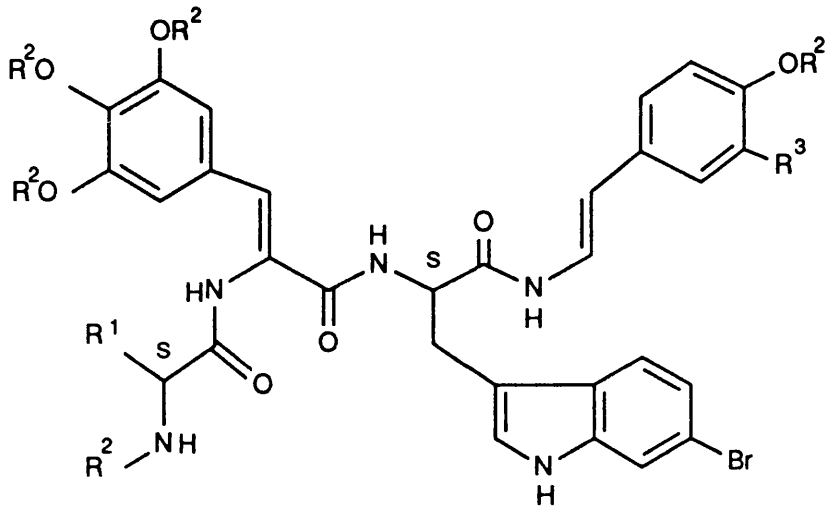
(c)*Tunichrome B-1 and celenamides (A-D).*- These two compounds are examples of marine metabolites. Tunichrome B-1 (**14**) is a yellow blood pigment isolated from the tunicate *Ascidia nigra* [42-44]. It is an extraordinarily oxygen sensitive compound which is thought to effect the ability of the tunicate to bind vanadium (III) or vanadium (IV) at physiological pH. Its structure is derived from three (3,4,5-trihydroxyphenyl)-alanine units and its reductive properties are thought due to the presence of the pyrogallol moiety [45].



Tunichrome B-1 (14)

The celenamides A-D were isolated in the form of their peracetyl derivatives from the sponge *cliona celata* and their structures have been elucidated [46,47]. The compounds are closely related to cyclopeptide alkaloids of plant origin and belong to the "linear peptide alkaloid" family. The peracetylcelenamides A-C (**15-17**) contain, in addition to

a styrylamine moiety, a 6-bromotryptophan unit; whereas peracetylcelenamide D contains instead 3,4,5-triacetoxy-α,β- dehydrophenylalanine.



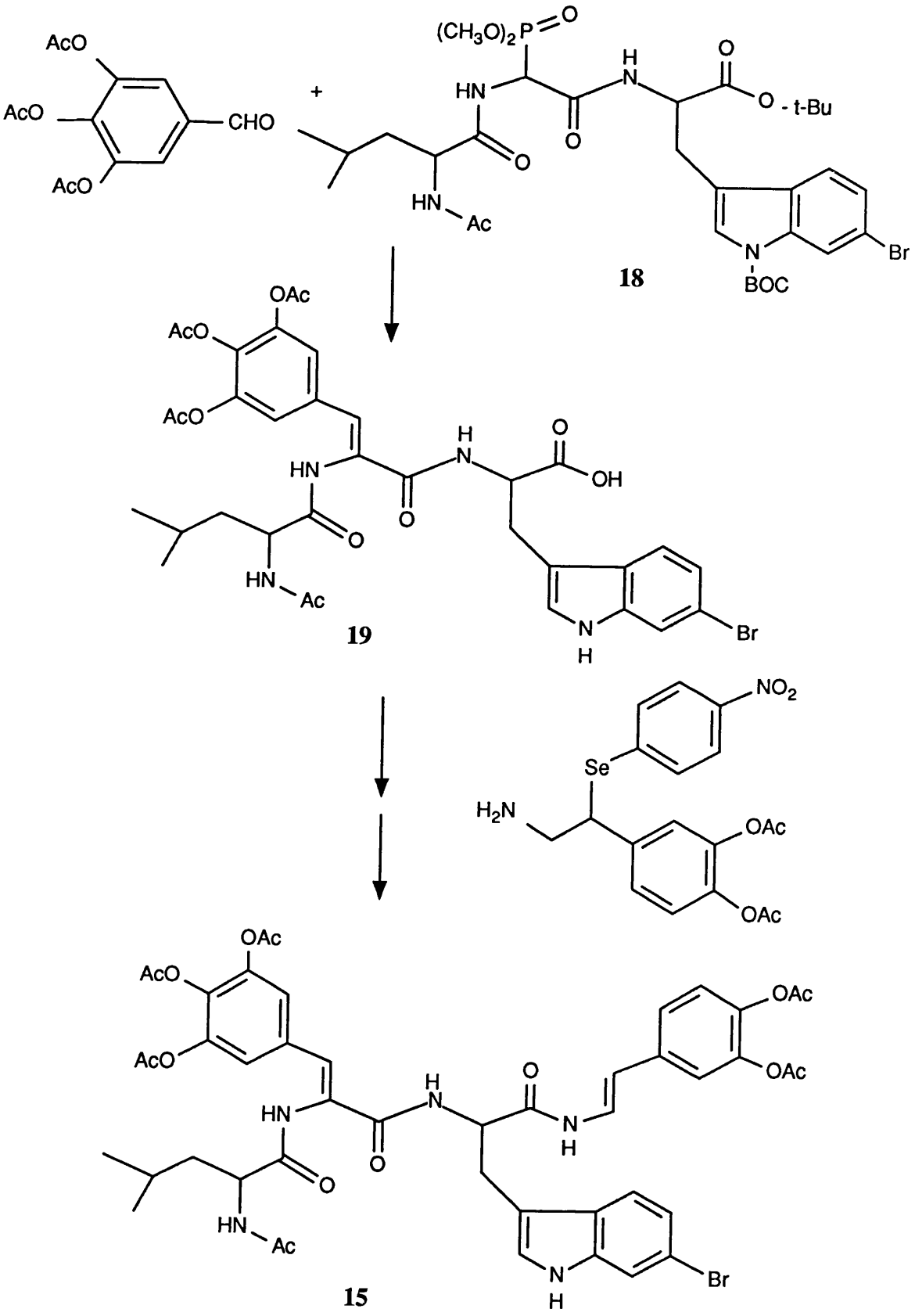
Peracetylcelenamides A-C (15-17)

Table 4

	R^1	R^2	R^3
15	*i*-C_4H_9	Ac	OAc
16	*i*-C_3H_7	Ac	OAc
17	*i*-C_4H_9	Ac	H

The total synthesis of hexaacetylcelenamide A (**15**) has been reported [48,49]. The key step in this synthesis is the condensation of the triacetoxybenzaldehyde with the phosphoryltripeptide (**18**) to give the dehydropeptide (**19**). The (Z)-isomer was separated from the E/Z mixture and taken on to the desired product (**15**) *via* a subsequent selenoxide elimination (Scheme 4).

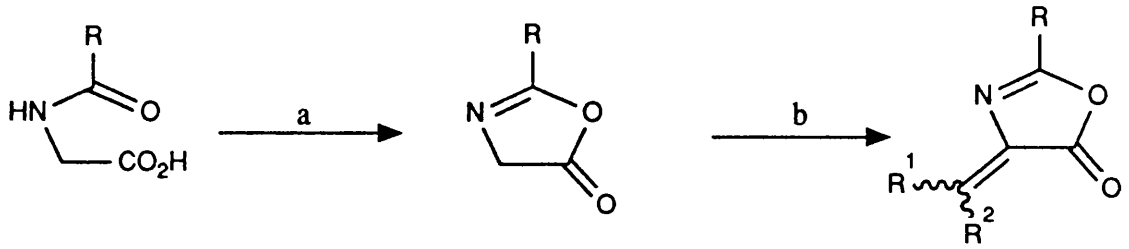
Scheme 4


AcO
AcO
AcO
CHO
+
(CH3O)2P=O
HN
H
N
O
O - t-Bu
O
HN
Ac
O
N
BOC
Br
18
OAc
AcO
AcO
HN
H
N
O
OH
O
O
HN
Ac
N
H
Br
19
NO2
Se
H2N
OAc
OAc
OAc
AcO
AcO
HN
H
N
O
N
H
OAc
OAc
O
HN
Ac
N
H
Br
15

Synthesis of α,β-Dehydroamino Acid and Derivatives

Use of oxazolones.-α,β-Dehydroamino acids are labile substances prone to isomerisation and hydrolysis. As a consequence, they are generally prepared as their N-acyl derivatives and sometimes as carboxylic esters or amides. The earliest work leading to the synthesis of dehydroamino acid derivatives came from the discovery by Erlenmeyer that aldehydes and ketones condense with oxazolones(**20**)(old name azlactones) in the presence of a base, to give an "unsaturated oxazolone" (**21**) [50] (Scheme 5).

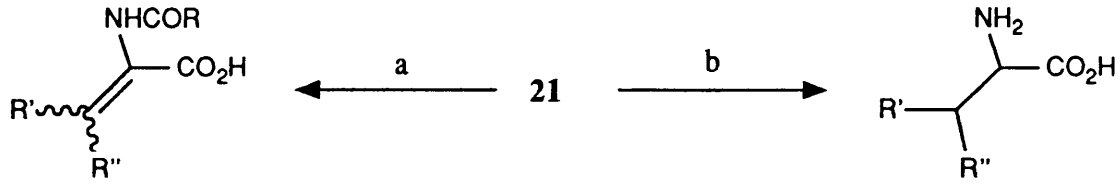
Scheme 5



20 **21**

Reagents : (a) Ac_2O, Δ ; (b) R^1COR^2 , base

Scheme 6



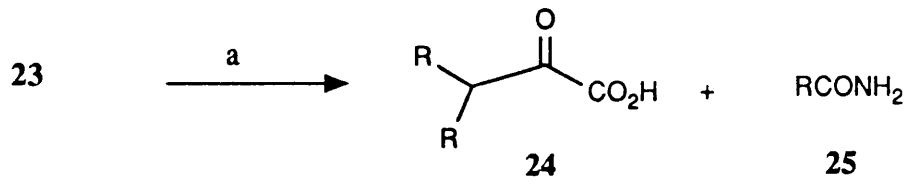
23 **22**

Reagents : (a) H^+ , H_2O ; (b) i) H_2 / cat. , ii) H_2O

Originally, the oxazolones (**21**) were hydrogenated to remove the double bond and the ring was opened hydrolytically to give the amino acid (**22**). This constituted a new synthesis of amino acids. If, however, **21** was carefully hydrolysed using either acidic or basic catalysis, a dehydroamino acid derivative (**23**) could be obtained (Scheme 6).

This method has been used extensively over the years. The Erlenmeyer condensation gives the best results when aromatic aldehydes are used; ketones and aliphatic aldehydes generally give poor yields of the unsaturated oxazolone. The N-acyl dehydroamino acid (**23**) can be coupled with other amino acids and peptides to form N-terminal dehydropeptides, but attempted hydrolytic deacylation yields the corresponding α-keto acid (**24**) and the amide (**25**). Thus **23** cannot be used to extend a peptide chain towards the amino terminal (Scheme 7).

Scheme 7

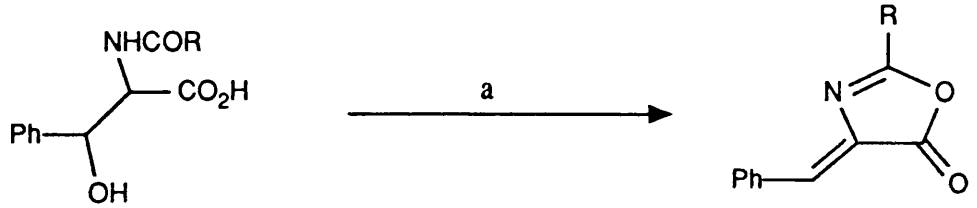


24 **25**

Reagents : (a) H^+ , H_2O

A second synthesis of oxazolones of the type **21** was reported by Bergmann [8,51], in which a phenylserine derivative (**26**) was oxazolonized in acetic anhydride to give **21** (R=H) spontaneously (Scheme 8).

Scheme 8



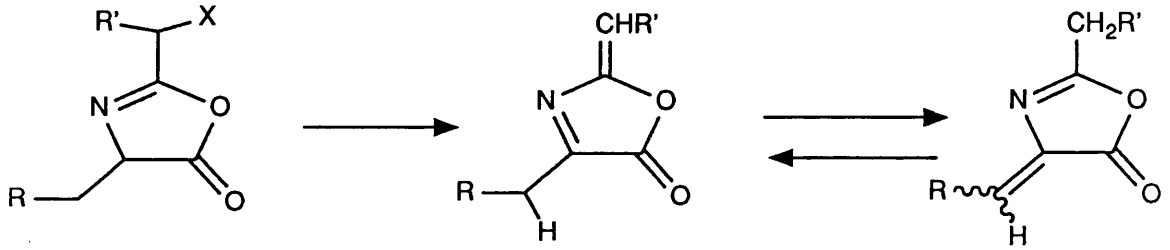
26 **21**

Reagents : (a) Ac_2O

In this case, the amino acid is already oxidised at the β position and elimination of the hydroxyl function, as the acetate, generated the double bond. If R in **26** corresponds to

an amino acid moiety, then **21** is a dipeptide oxazolone and can be used to synthesize dehydropeptides. This approach was used by Stoll *et al* [52]. In 1926 Bergmann [53] reported that halooxazolones of the type **27** undergo spontaneous dehydrohalogenation giving a "pseudo oxazolone" (**28**) that equilibrates with its tautomer (**29**) (Scheme 9).

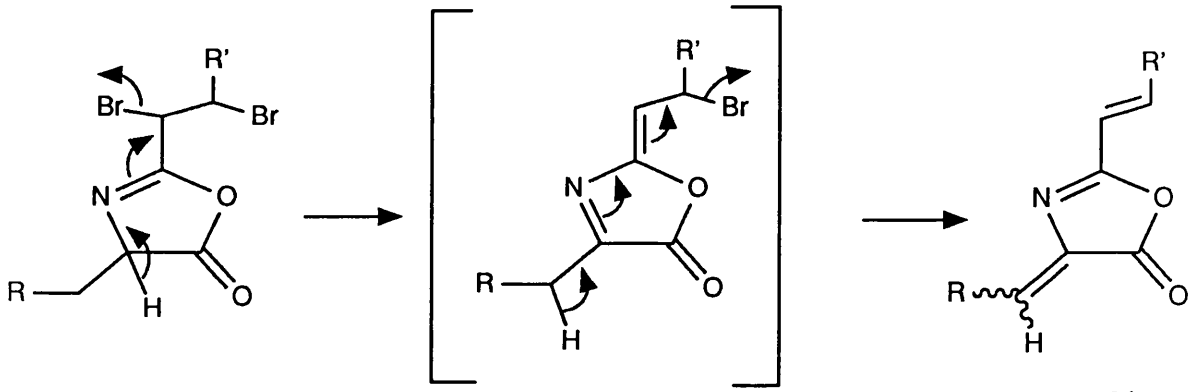
Scheme 9



27 (X = Cl , Br) **28** **29**

Stammer, more recently, showed [54] that when a dibromooxazolone (**30**) is taken, instead of **27**, a double dehydrohalogenation results giving an oxazolone (**31**) that has the desired double bond in the 4 position (Scheme 10).

Scheme 10



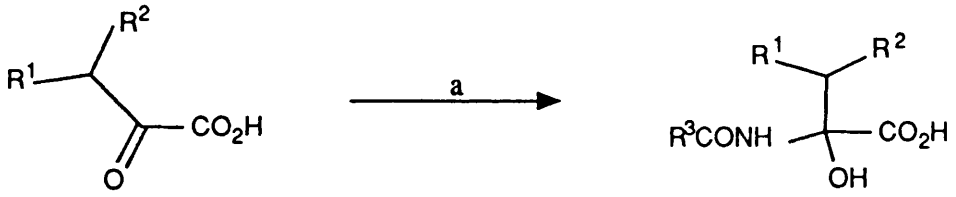
30 **31**

Oxazolones of the type **31** are, as in the case of **21**, only useful in the preparation of N-terminal dehydropeptides. The aromatic dehydroamino acid derivatives prepared by this, or by any of the other methods discussed, all have the (Z)-configuration.

By the early 1960's few dehydropeptides containing other dehydroamino acids had been prepared because of the instability of oxazolones with alkyl substituents. Since then, however, there has been a revival of interest in the synthesis of dehydroamino acids owing to the recent discovery of many natural products containing these unsaturated residues. As a consequence of this, more facile and effective methods of preparation have been developed.

β-*Elimination reactions*.- The elimination of water from both α- and β-hydroxy-α-amino acid derivatives is an important method for the preparation of dehydroamino acids and peptides. In the case of the α-hydroxy-α-amino derivatives (**32**) a wide variety of amino acids are accessible *via* the condensation of an α-keto acid (**24**) with an amide (Scheme 11).

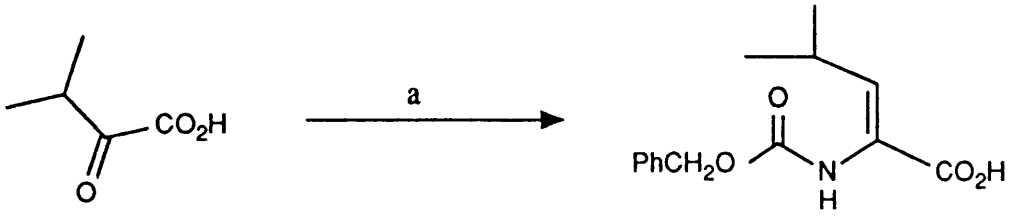
Scheme 11



24 **32**

Reagents : (a) R^3CONH_2

Scheme 12



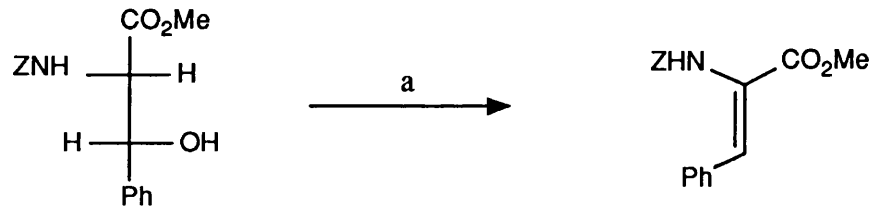
33 Z- stereochemistry

Reagents : (a) $PhCH_2OCONH_2$, *p* - TsOH

When benzyl carbamate was used as an amide component for the α-keto acid (**33**), the elimination of water was facilitated *via* the addition of a catalytic amount of *p*-toluenesulphonic acid to generate dehydroleucine having (Z)-stereochemistry [55] (Scheme 12).

Use of the dehydrating agents *e.g.* disuccinimido carbonate (DSC) [56,57], N,N-carbonyldiimidazole [58] or a base and acetic anhydride [59], has been recommended for the dehydration of natural β-hydroxy-α-amino acids, serine, threonine and phenylserine [60]. In the case of phenylserine it was found that the *threo* isomer (**34**) gives only the Z-dehydrophenylalanine (Δ^{Z}Phe) with DSC (Scheme 13).

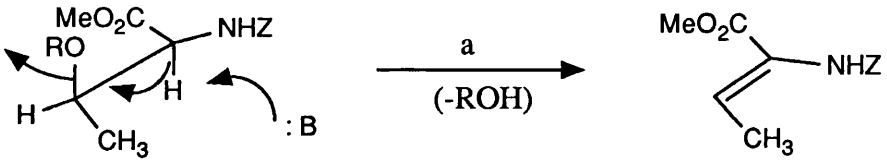
Scheme 13



34

Reagents : (a) DSC , Et_3N

Scheme 14



threo - form (Z) - isomer

where R= Ts , Z= CBZ

Reagents : (a) base

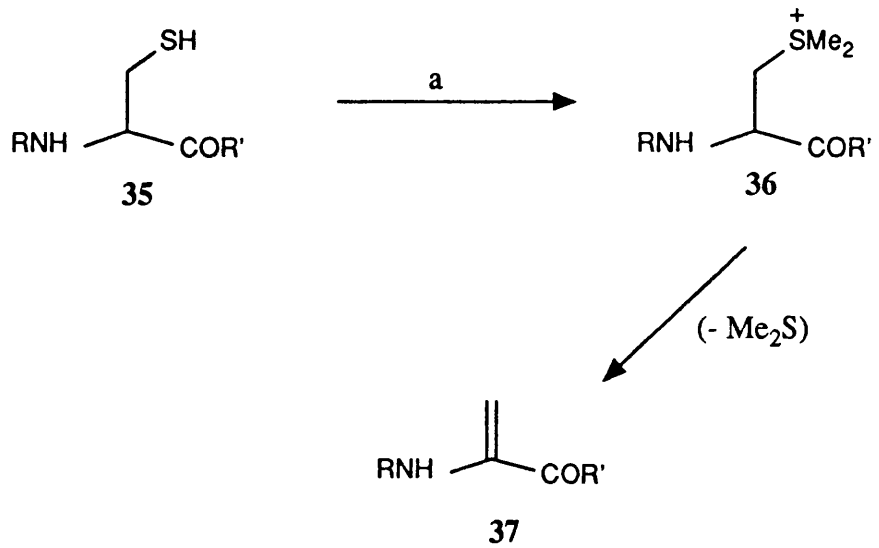
Lee *et al* [61] have similarly confirmed these stereochemical results with the O-tosyl

derivatives of threonine. They obtained the (Z)- and (E)- dehydrophenylanine isomers from the threo and erythro forms respectively. These eliminations are thought to go *via* a *trans* E_2- type mechanism (Scheme 14).

It should be noted, however, that competitive side reactions such as aziridine formation may occur with certain O-tosylthreonine peptides on treatment with base [62]. Also, isourea-mediated β-elimination methods of threonine peptides [63] involving carbodiimide and copper(I)chloride catalysis have yielded a mixture of (Z)- and (E)-isomers in a 2:3 ratio.

Dehydration of the β-hydroxy-α-amino acid can also be accomplished by use of the Mitsonubu reagent, but the structure and reaction conditions are crucial in determining whether a dehydroamino acid or a aziridine results [64]. In the preparation of a dehydroalanine unit one of the simplest methods is the conversion of a cysteine moiety (**35**) to the S,S-dimethylsulfonium salt (**36**) with methyl iodide and subsequent β-elimination with base [65-67] (Scheme 15).

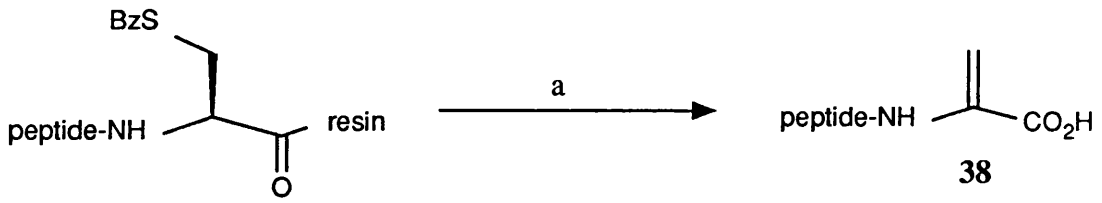
Scheme 15



Reagents : (a) MeI , base.

Eliminations from the corresponding selenium derivatives of **36** [68,69] and S-phosphinothionylcysteines have also been suggested [70]. Similarly, Nomoto *et al* [71] reported the direct preparation of ΔAla residue from a α,β-diaminopropionic acid residue, mediated by a Hofmann rearrangement, utilizing methyl iodide and potassium bicarbonate. Thiol elimination (S-benzyl) has also been achieved [72] on the attempted release of a C-terminal Cys peptide from the bound resin, with hydrofluoric acid, resulting in the formation of a ΔAla peptide (**38**)(Scheme 16).

Scheme 16



Reagents : (a) HF, 25^{0}C .

The cleavage of water from N-hydroxylamino acids [73] proceeds almost quantitatively [74] and is to be recommended when the appropriate substrates are readily available.

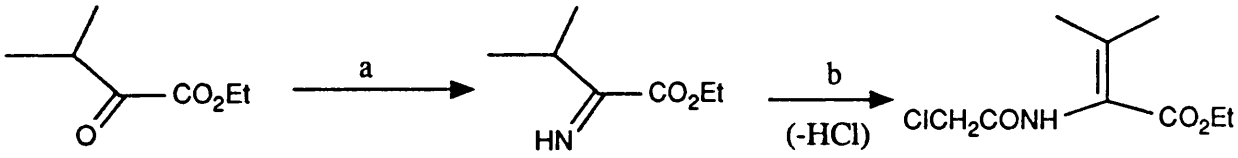
N-Chlorination and dehydrochlorination.- The N-chlorination of amino acid esters and N-acylamino acid esters with *t*-butylhypochlorite and subsequent elimination of hydrogen chloride has been reported [75-77]. Initially a mixture of the imine (**40**) and the enamine (**41**) is obtained from the amino acid ester (**39**). Treatment of this mixture with hydrogen chloride in ether gives rise to the enamine hydrochloride.

The N-chloroamide (**42**), initially formed from the acyl amino acid ester, reacts depending on the solvent and base used, to give either the α-acylamino-α-alkoxycarboxylate (**43**) or the acylimino compound (**44**). Treatment of the former with acid, or of the latter with a strong base, results in the formation of the

N-acyldehydroamino acid derivative (**45**). This reaction sequence has been employed frequently in the last few years [78-82] and can also be performed with peptides [79] (Scheme 17, see page 25).

An alternative preparation of the imine (**46**) was reported by Shin *et al* [55] who condensed an α-keto ester with triphenyl phosphine imine. The imine (**46**) was converted to the dehydroamino ester derivative (**47**) *via* subsequent acylation with chloroacetyl chloride (Scheme 18).

Scheme 18

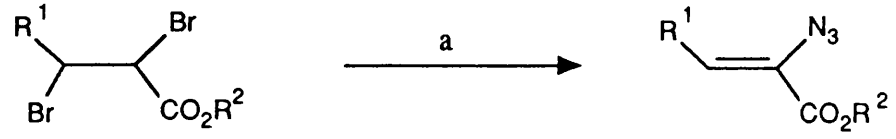


46 **47**

Reagents : (a) $Ph_3P{=}NH$; (b) $ClCH_2COCl$

From azido compounds and aziridines.- α-Azidoacrylates (**49**) are easily accessible from bromocarboxylates (**48**) through treatment with sodium azide [83] (Scheme 19).

Scheme 19

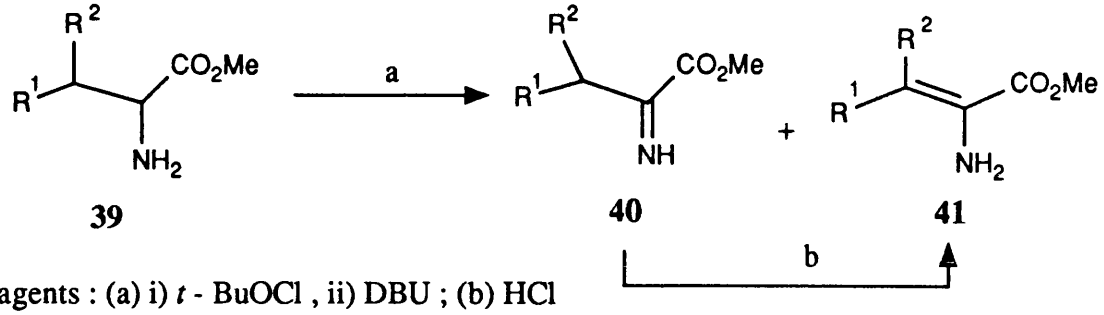


48 **49**

Reagents : (a) NaN_3 , DMF

In the presence of phosphites or phosphinites, phosphinimines (**50**) are formed, which rearrange to give the (Z)-phosphoric amides or -phosphinic amides respectively, of the dehydroamino acid (**51**) [84] (Scheme 20, see page 26).

Scheme 17

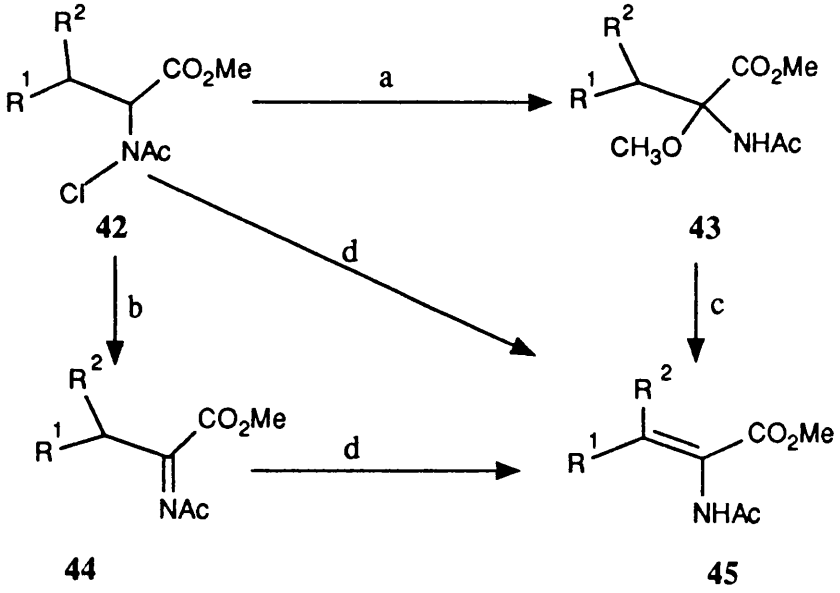


39 40 41

Reagents : (a) i) *t* - BuOCl , ii) DBU ; (b) HCl

Table 5

	R^1	R^2	Yield (%)
42A	CH_3	CH_3	80
B	H	*i* - C_3H_7	63
C	CH_3	C_2H_5	75



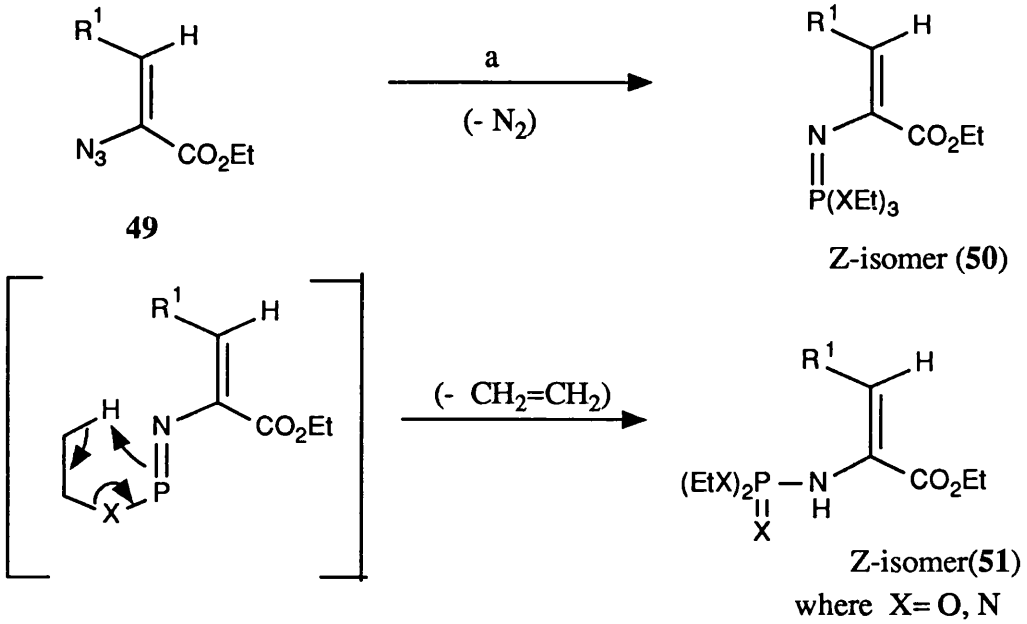
42 43 44 45

Reagents : (a) CH_3ONa; (b) Et_3N; (c) HCl; (d) DBU

Table 6

	R^1	R^2	Yield(%)
45A	CH_3	CH_3	70
B	H	*i* - C_3H_7	65
C	H	CH_3	50

Scheme 20



49

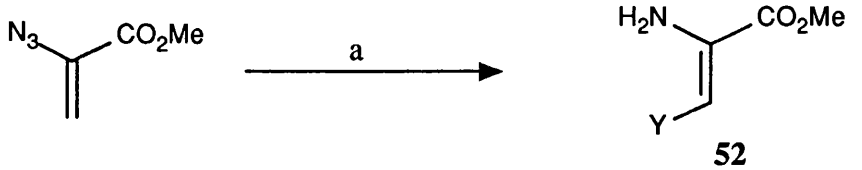
Z-isomer (**50**)

Z-isomer(**51**)
where X= O, N

Reagents : (a) $P(XR)_3$, benzene

Various thiols and alcohols undergo addition to α-azidoacrylates in alkaline solution. The β-phenylthio- and β-alkoxy-α-azidocarboxylates formed are not isolated, but undergo cleavage of nitrogen under the action of alkoxides, to give the corresponding β-substituted dehydroamino acid (**52**) [85] (Scheme 21).

Scheme 21



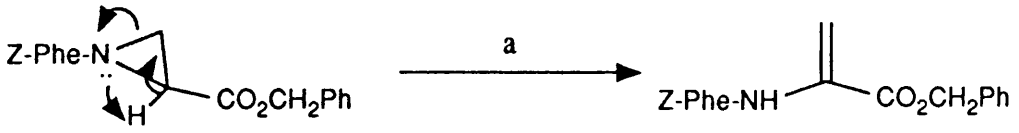
52

where Y= PhS , EtO

Reagents : (a) i) Y-H ; ii) NaOEt

It has been found that in the presence of tertiary amines, (Z)-Phe-Azy-OBzl dipeptides (**53**) undergo rearrangement to give (Z)-Phe-ΔAla-OBzl (**54**) [86] (Scheme 22).

Scheme 22



53 **54**

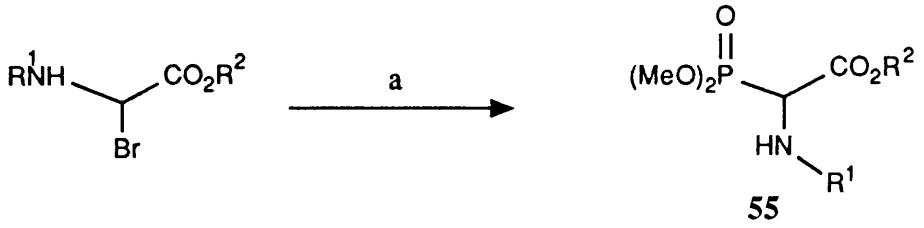
where Z= $PhCH_2OCO$

Reagents : (a) Et_3N , CH_2Cl_2

This reaction has been applied to peptides containing aziridinecarboxylic acids [87] and the reaction was found to proceed most favourably in the presence of sodium iodide as catalyst [88].

From phosphorylglycine esters.- N-acyldialkoxyphosphorylglycine esters (**55**) have been previously used in the synthesis of dehydroamino acid esters [89-90]. The glycine esters (**55**) can be prepared *via* the treatment of acylaminobromoacetates with phosphites [91] (Scheme 23).

Scheme 23

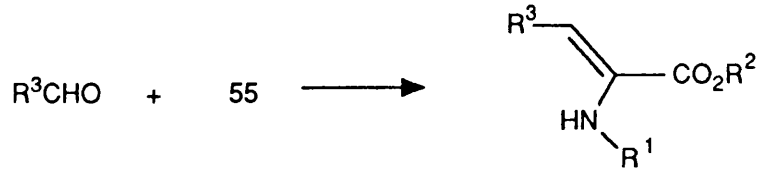


55

Reagents : (a) $P(OMe)_3$

The dehydroamino acid esters are afforded *via* the condensation of N-acyldialkoxyphosphorylglycine esters (**55**) with aldehydes in the presence of base. If potassium *t*-butoxide is used as base, dehydroamino acid esters, having (Z)-stereochemistry, are preferentially formed (Scheme 24).

Scheme 24

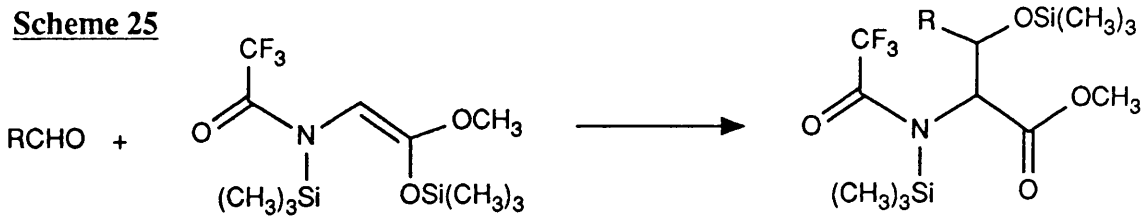


Z- stereochemistry

Dimethoxyphosphoryl glycine can also be incorporated into peptides without difficulty. The condensation of the dimethoxyphosphoryl peptide with aldehydes leads directly to dehydropeptides [91].

From glycine derivatives.-Aromatic and heterocyclic aldehydes have been condensed with trimethylsilyl(trifluroacetyl)amino ketene methyl trimethylsilyl acetal (**56**) to furnish 2-trifluroacetyl amino-3-trimethylsiloxycarboxylates (**57**). These were subsequently converted to the (Z)-dehydroamino acid esters (**58**) in 60-85% overall yield, by cleavage of trimethylsilanol (Scheme 25) [92,93].

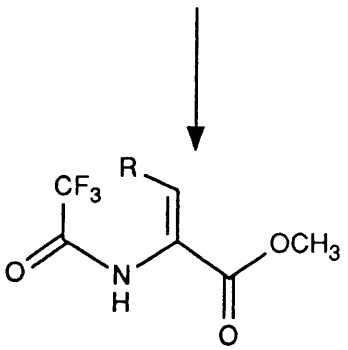
Scheme 25



56 **57**

Table 7

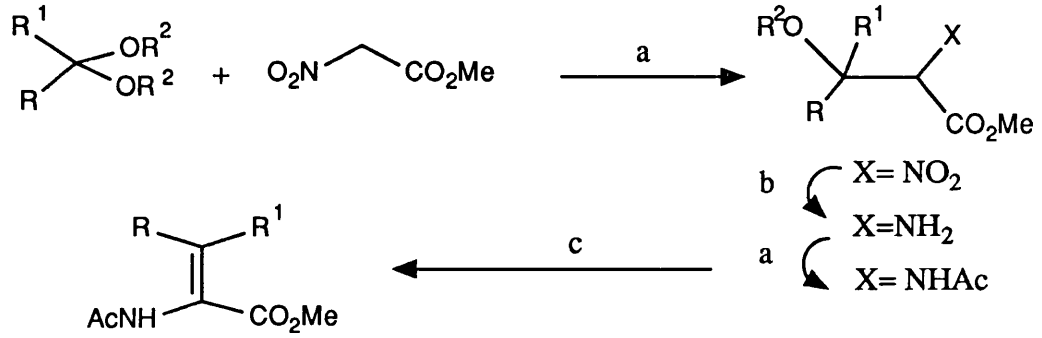
R	**57** : Yield (%)	**58** : Yield (%)
3,4-$(CH_3O)_2C_6H_5$	82	93
2-thienyl	82	83
2-furyl	81	94
1-napthyl	86	85



Z-isomer **58**

The synthesis of N-acyl-dehydroamino acid esters *via* the condensation of acetals with nitroacetates in acetic anhydride was reported by Kochetkov in 1982 [94]. Initially the β-alkoxy-α-nitroacetates were formed, then subsequent reduction and acylation, followed by dehydration, yielded the desired compound (**59**) (Scheme 26).

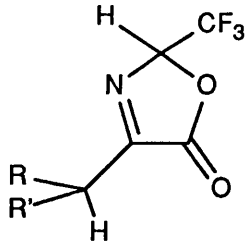
Scheme 26



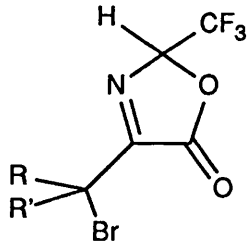
59

Reagents : (a) Ac_2O ; (b) raney nickel ; (c) NaOMe , MeOH

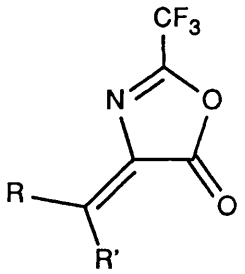
From modified oxazolones.- Previous work [95] has shown that amino acids could be converted to pseudo-oxazolones (**60**) with trifluroacetic anhydride, and that these could be readily brominated in the β-position to afford **61**. It has since been found that these bromo compounds (**61**) readily eliminate hydrogen bromide to afford the unsaturated oxazolone (**62**) [96,97].



60



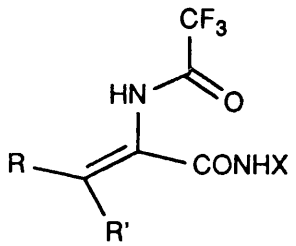
61



62

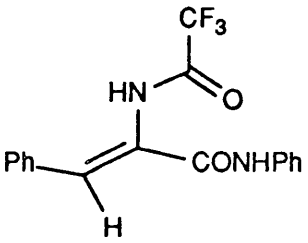
Both aliphatic (Abu, Ala, Val, Leu, Ile) and aromatic (Phe) amino acids readily formed the unsaturated oxazolone (**62**) with (Z)-configurations favoured. These compounds

were found to be very reactive towards nucleophiles giving the acyl derivative (**63**) in good yield. The anilide (**64**) could readily be deblocked by ammonia to give the free dehydrophenylalanine anilide (**65**). Unfortunately the amino group in (**65**) was too inert to undergo coupling reactions.

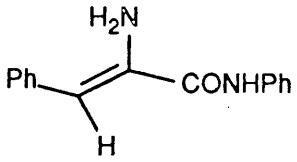


where X=Nu

63

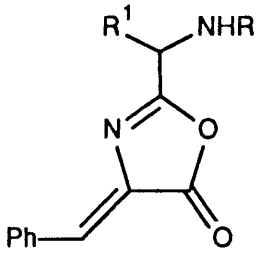


64

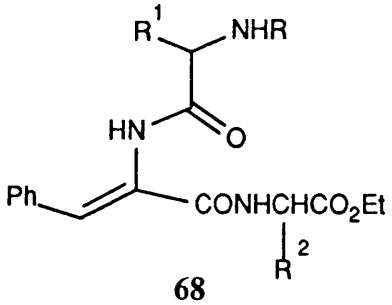


65

More recently it has been shown [98,99] that the N-phthaloylglyclphenylalanine oxazolone, a dipeptide oxazolone, could be directly oxidised using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to give the unsaturated oxazolone (**67**, R^1 = H, R = Phth). This reaction was extended to include several N-terminal amino acids (Leu, Phe, Gly) and C-terminal amino acids (Phe,Tyr, Trp). Hence the difficult step of the coupling of the enamine nitrogen has been avoided in synthesising dehydrooligopeptides (**68**).

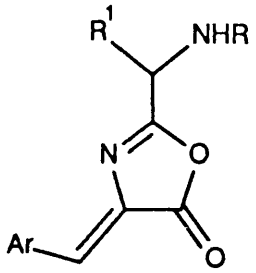


67



68

The Erlenmeyer synthesis has recently been performed upon C-terminal glycine peptides *via* the condensation with aromatic aldehydes.

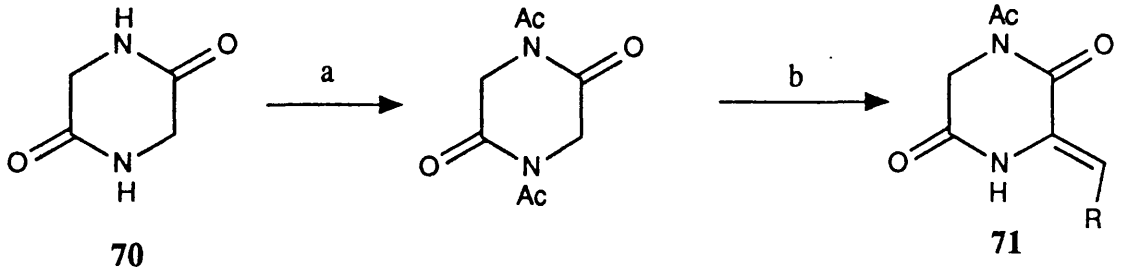


69

The (Z)-dehydrooxazolones formed (**69**), have been cleaved with amino acid salts to give rise to tripeptides possessing a central dehydroamino acid. Tri- and tetrapeptides, even with two dehydrophenylalanine units, have been synthesized [100-106]. The disadvantage of the Erlenmeyer synthesis is that drastic conditions are usually required.

From piperazin-2,5-dione.-Piperazin-2,5-dione (**70**) containing glycine residues can be condensed with aldehydes at the α-methylene site [107,108]. This approach has been adapted by Gallina *et al* [109] *via* the advance activation of the α-methylene group by N-acylation to afford the alkylidene/arylidene (**71**)(Scheme 27).

Scheme 27



70 **71**

Reagents : (a) Ac_2O ; (b) RCHO , *t* - BuOK

Utilizing this procedure for piperazin-2,5-dione containing L-glycine and L-amino acids, Kanmera *et al* [110] prepared a variety of dehydroamino acids (ΔAbu, ΔVal, ΔPhe, ΔApp and ΔTrp).

It should be noted that many dehydropiperazin-2,5-diones have been found in bacterial metabolites. Their preparation and incorporation into various metabolites has been reviewed by Schmidt *et al* [111].

The isomerisation of (Z) → (E) alkenes.- The equilibration of (Z)-dehydrooxazolone to its (E)-geometrical isomer was generally performed either by the addition of hydrogen bromide or phosphoric acid, followed by elimination [112,113], or by photoequilibration. For other unsaturated compounds photoequilibration was usually employed.

Determination of (Z)- and (E)- configurations.- When two geometric isomers of the α,β-double bond in dehydroamino acids are possible (ΔAbu, ΔLeu, ΔPhe, *etc.*) it is important to determine which stereochemistry results: whether Z, E or Z/E mixed configuration. Such a structural examination can be made directly by X-ray analysis or by ^{1}H- and ^{13}C-NMR spectroscopy. The following empirical regularities have been developed in recent years:-

(a) A difference in the chemical shift (Δδ) of the β-vinyl and amide protons [114] and the β-alkyl proton [115,116]. The (E)-isomer appears 0.2 - 0.7 ppm downfield from the (Z)-isomer.

(b) A change in the chemical shift (Δδ) of the vinyl proton when measured in $CDCl_3$ and trifluroacetic acid (TFA) [115] : (Z)-isomer Δδ = 0.34 - 0.54 ppm downfield shift; (E)-isomer, Δδ = 0.18 - 0.32 ppm upfield shift.

(c) A comparison of the chemical shifts of the vinyl proton before and after N-methylation [115,117]; (Z)-isomer, almost no change; (E)-isomer, 0.7 - 0.9 ppm upfield shift.

(d) A difference in the vicinal coupling constant (J_{CH}) between the carbonyl carbon and the vinyl proton in the coupled nuclear Overhauser enhanced (n.O.e.) ^{13}C-NMR spectrum [118]: (Z)-isomer, J ≈ 5 Hz; (E)-isomer, J ≈ 10 Hz.

(e) A differential nuclear Overhauser effect between the vinyl and amide protons [119] : (Z)-isomer, n.O.e.= 0%; (E)-isomer, n.O.e.= 26-37%.

Rich and Mathiaparanam [120], in 1974, reported the U.V. absorption peak of the ΔPhe peptides in assigning the (Z)-configuration (ϵ = 18,400 at λ= 276 nm) and the (E)-configuration (ϵ = 9080 at λ= 282 nm). Such a difference in the intensity of the peak was not observed for the isomers of N-blocked ΔPhe-OH and its esters [121,122]. Since spectroscopic pictures are often sensitive to local factors around the α,β-double bond, the use of general combined measurements is recommended for structural examinations.

Coupling Reactions of α,β-Dehydroamino Acid Derivatives

When α,β-dehydroamino acid esters are used as the amine component in peptide couplings, the coupling tends to be low yielding. This is because the amine group of an enamino function is less nucleophilic than a normal amine, owing to it being in equilibrium with its tautomeric imino form. Even though such coupling methods as those involving an acid chloride [55,123] or mixed anhydrides [124] have been used in the acylation of enamino acid esters. Shin *et al* [125] has shown that the decyclohexylcarbodiimide (DCC) coupling of the dipeptides (Z)-Ser-OH and (Z)-Thr-OH with several α,β-dehydroamino acid esters gave only a 20-50% yield of the desired dipeptides. The water soluble carbodiimide (EDC)/HOBt, however, was found to be an effective method for the coupling of BOC-Phe-OH and H-ΔLeu-OBzl in 50% yield. [126,127].

When N-blocked α,β-dehydroamino acids are used as acid components, the usual coupling procedures are applicable, for example, mixed anhydride [76,99], N-carboxyanhydride (NCA) [128] or DCC [125] methods.

Peptides Containing α,β-Dehydroamino Acids

Over the years there has been increased interest in the synthesis of natural dehydropeptides and also of unsaturated analogues of peptide hormones.

Various natural products have been synthesised including the phytotoxic metabolite Tentoxin, achieved by Rich and co-workers [120,129,130], which causes chlorosis in germinating seedlings of many flowering plants [131]. It is a cyclotetrapeptide containing a single (Z)-N-methyldehydrophenylalanine unit.

A considerable number of these natural products are biologically active and most of them possess antibiotic properties. The presence of these dehydroamino acids within peptides is thought to be as intermediates in the interchange of natural L-amino acids into their D-enantiomeric form, since these D-isomers cannot be incorporated directly into the peptide chain [132]. This explains why a great number of microbial peptides having antibiotic activity contain both α,β-unsaturated and D-amino acid residues. Incorporation of a dehydroamino acid unit into the peptide decreases its conformational flexibility and has been found to have a stabilising influence upon a type II β-turn (Found $\phi \approx 60°$, $\psi \approx 0°$; typical value $\phi = 80°$, $\psi = 0°$) [133,134]. This phenomenon has been used in analogues of biologically active peptides to reduce their enzymatic degradation [135]. It has recently been found [136] that if a peptide possesses two dehydrophenylalanine moieties adjacent to each other as in N-Ac-ΔPhe-ΔPhe-Gly then a β-pleated sheet structure results. Whereas, if they are separated by either a glycine or the more hindered valine residue, as in Boc-Ala-ΔPhe-Gly-ΔPhe-Gly-OMe and Ac-ΔPhe-L-Val-ΔPhe-NHMe respectively, then a 3_{10} helix is formed (Found $\phi = -58°$,

$\psi = -23°$; typical value $\phi = -60°$, $\psi = -30°$) [137].

Peptide mimetic studies have led to the synthesis of various biologically active peptides, in which an α-amino acid residue has been replaced by its unsaturated equivalent. In 1981, Fischer *et al* [138] synthesized the unsaturated analogues of the vasodilator peptide hormone bradykinin (Bk) (**72**). (ΔPhe^5)Bk showed a high biological activity in its blood pressure-lowering effects, being 23-fold more potent than Bk.

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

72

Enkephalin (**73**), which is found in the brain as an opiate-like neuropeptide, has been synthesized in a derivatised form by English and Stammer [139]. They reported the synthesis of a highly potent (D-Ala^2, ΔPhe^4, Met^5)-enkephalin amide which was 5-fold more potent than the saturated analogue.

H-Tyr-Gly-Gly-Phe-Leu(or Met)-OH

73

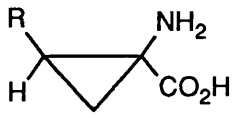
Also, a series of unsaturated (D-Ala^2, Leu^5)-enkephalin analogues (ΔAla^2)-, (D-Ala^2, ΔAla^3)-, (D-Ala^2, ΔPhe^4)- and (D-Ala^2, ΔLeu^5)-enkephalins were prepared. These analogues exhibited almost the full receptor activity [140]. It has been shown that for (ΔPhe^4)- and (ΔLeu^5)-enkephalins the δ-enkephalin receptor selectivity has remained unchanged. Furthermore it has been suggested that the phenyl ring of ΔPhe^4, oriented in the (Z)-configuration, is important for the interaction with the δ-receptors [141].

1.4 2,3-Methanoamino Acids and Peptides

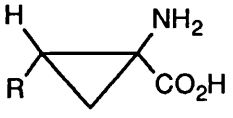
Definition and Nomenclature

The nomenclature of the title compounds will be named and treated as modified amino acids. The carboxyl carbon atom is given the number one (**1**) rather than the quaternary ring carbon atom which is C^{α} in the common nomenclature system. The individual amino acids will be referred to as 2,3-methano- or 3,4-methanoamino acids, depending upon the position of the cyclopropane ring on the amino and carbon chain. Here only the 2,3-methanoamino acid will be discussed.

The symbol ∇^{Z} or ∇^{E} prefixed to the abbreviation for an amino acid residue, as in ∇^{Z}Phe, means the (Z)-diastereomer of 2,3-methano- or cyclopropane-phenylalanine. It is used here only when the methanoamino acid appears in a peptide chain. The Δ^{Z} symbol indicates a dehydroamino acid as in Δ^{Z}Phe, meaning (Z)-2,3-dehydro-phenylalanine. The only exceptional case to this terminology will be the simplest cyclopropane amino acid, 1-aminocyclopropane-1-carboxylic acid (ACC) which might be called 2,3-methanoalanine, but is well known in the literature as ACC.



Z- isomer



E- isomer

Except for the 2,3-methano analogues of proline and valine, each protein amino acid cyclopropane analogue exists in diastereomeric (E)- and (Z)- forms in which the characteristic functionality at the β-carbon atom of the specific amino acid is *cis* to the carboxyl or to the amino function respectively, Of course each of these diastereomers

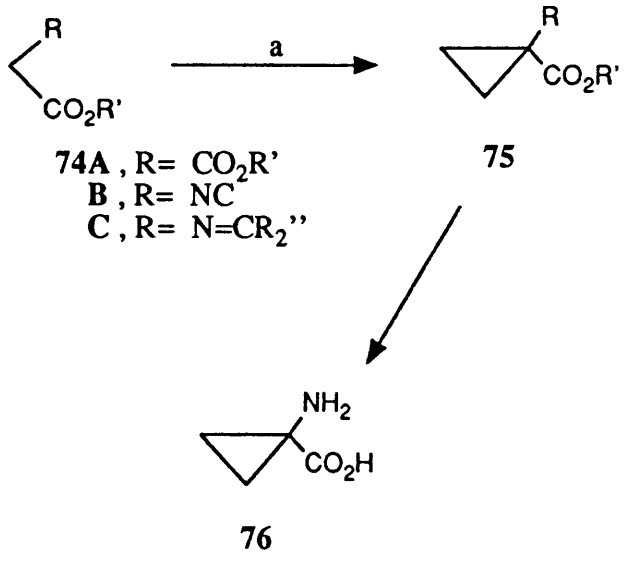
consists of an enantiomeric pair, as does 2,3-methanoproline and valine.

Naturally Occurring 2,3-Methanoamino Acids

(a)*1-Aminocyclopropane-1-carboxylic acids.*- 1-Aminocyclopropane-1-carboxylic acid (ACC) was first isolated in 1957 from ripe cider apples and perry pears by Burroughs [157] and from ripe cowberries by Vahatalo and Virtanen [158]. It was later found to be the key intermediate in the biosynthesis of ethylene from methionine [159] in plants. Subsequently it has been of considerable interest to both plant biologists and chemists alike.

There have been many different approaches to the synthesis of ACC. One of the earliest and most straightforward procedures involves the alkylation of a glycine derivative or congener with ethylene dibromide or its equivalent (Scheme 28).

Scheme 28

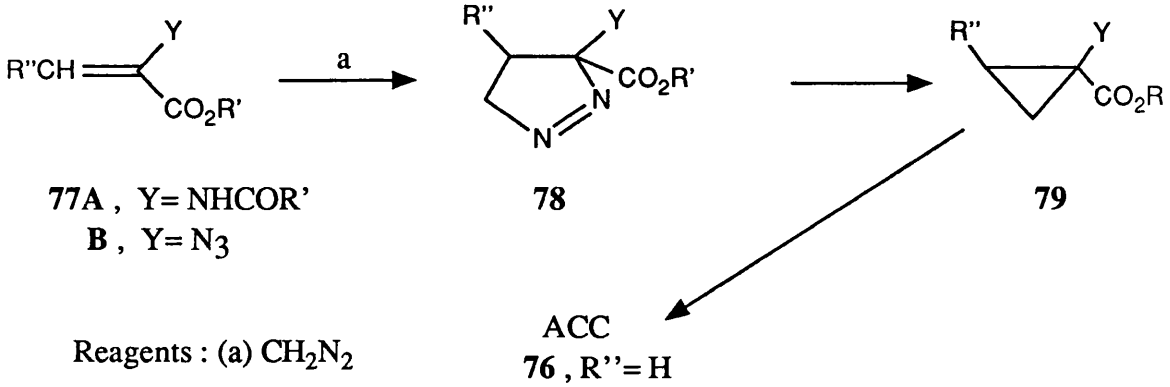


Reagents : (a) $BrCH_2CH_2Br$, base

The first synthesis, by Ingold [160], started with the diester (**74A**, R′=Et), followed by the conversion of one ester function into an amino group. This was achieved by base

rearrangement of the bis-N-bromoamide into a spirohydantoin which was hydrolysed to give the free amino acid (**76**). This method has been repeated by others [161-163]. The more elegant use of the isonitrile (**74B**) prepared from N-formylglycine ester, obviates the need for the amide rearrangement, since simple hydrolysis of the isonitrile affords the amino group directly [164]. This approach was used to prepare specific ring deuterated ACC isomers for use in biological studies [165,166]. The process is simplified even further by the use of the benzophenone Schiff base (**74C,** R″=Ph) [167] and its conversion into **76** is accomplished by phase-transfer catalysed cycloalkylation, followed by hydrolysis [168]. The synthesis of 2-methyl-ACC and various deuterated isomers was achieved on the starting material (**74A,** R′-t-butyl) for use in enzyme studies [169-170]. A second synthetic approach to **76** is the "diazo addition" method in which diazomethane is added to an α-substituted acrylic acid derivative (**77A,** R″=H) forming a pyrazoline (**78A,** R″=H) which can be converted into the desired cyclopropane (**76**) (Scheme 29).

Scheme 29

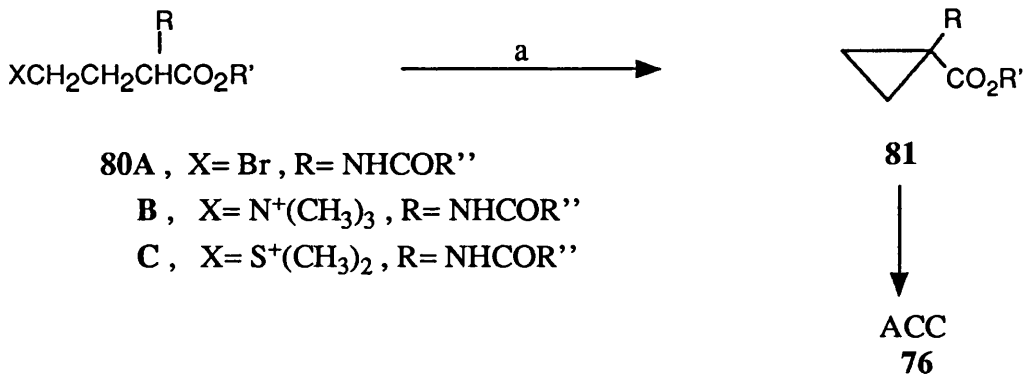


This method was first carried out by Bregovec and Jakovac [171], with R=R′=Me, who applied thermolysis conditions to convert the pyrazoline (**78A,** R″=H) into the cyclopropane (**79A,** R″=H). Alkyl and aryl substituted diazomethane give 5-substituted pyrazolines and, subsequently, 2-substituted-ACC derivatives [172]. It has been found that the final removal of the N-acyl group by vigorous acidic

hydrolysis, or hydrogenolysis in the case of the benzyloxycarbonyl blocking group, has been successful only when the 2-substituent is hydrogen or alkyl [173-174]. Although, when Y is an azido group (**77B**), the final step requires the reduction of the azido function [175] and is reported to be successful when R″=H, Me, Et and Ph.

A third type of ACC synthesis proceeds through the intramolecular cyclisation of a γ-substituted α-aminobutyric acid derivative or precursor (**80**) (Scheme 30).

Scheme 30



Reagents : (a) base

Use of a strong base on **80A**, R″=OBzl gave the cyclopropane (**81A** R″=OBzl) which was converted to **76** by hydrolysis [176-178]. Since the γ-carboxyl group of glutamic acid can be converted into a leaving group, glutamic acid can serve as the ultimate starting material for ACC [179]. The quaternary ammonium function [180] of **80B** and the dimethylsulfonium group [181] of **80C** can also replace the bromide as a leaving group in this cyclisation, giving good yields of various cyclopropane intermediates (**81**). Similarly, the cyclisation of γ-chloro-α-aminobutyronitrile with base gave ACC after hydrolysis [182].

ACC can also be prepared *via* the nitration of the α-carbanion formed from cyclopropanecarboxylic acid esters with *t*-butyllithium, followed by the reduction of

the intermediate nitro compound [183]. Interestingly, the intermediate nitro ester, having geminal electron accepting groups on the ring, is quite susceptible to ring opening by various nucleophiles [184], with the formation of γ-substituted α-nitro acids convertible into special amino acids. It has been recently demonstrated [185] that cyclopropanone acetals undergo conversion to 1,1-aminocyanocyclopropanes *via* the Strecker synthesis [186]. The subsequent hydrolysis and hydrogenolysis of which afforded ACC in good yield.

Optically active 2,2-dideutero-ACC were prepared *via* alkylation of the chiral intermediate, (R)-(-)-2,5-dimethoxy-3-benzyl-3-methyl-3,6-dihydropyrazine , used previously for the asymmetric synthesis of α-amino acids [187,188]. Their absolute configurations were determined *via* NMR studies [189-191].

The stereochemistry of the enzymatic decomposition of ACC to ethylene and its synthesis from S-adenosylmethionine (SAM) in plants has been studied in considerable detail. An early paper [192] showed that postclimateric apple slices and mungbean hypocotyls converted the (1R, 2S)-2-ethyl-ACC into but-1-ene 50-200 fold faster than they decomposed the other three stereoisomers of 2-ethyl-ACC. Further work with [1-^{14}C] ACC led to the conclusion that, in mungbeans [193], the carboxyl group yielded carbon dioxide and the quaternary carbon (C^{α}) was released as hydrogen cyanide: work with 1-azidocyclopropanecarboxylic acid and ^{13}C and ^{2}H labelled ACC led to the same conclusion [194-196]. A detailed mechanism [197] has been proposed and the reaction pathway has been subjected to semi-empirical molecular orbital calculations [198], which support the formation of radical intermediates. Surprisingly, it was found that enzymatic decomposition of stereospecifically deuterated ACC derivatives occurred non-stereospecifically; whilst chemical decomposition, using sodium hypochlorite, took place with reproducible retention of configuration [169,199]. These stereochemical results can be explained by involving cation radical and nitrene

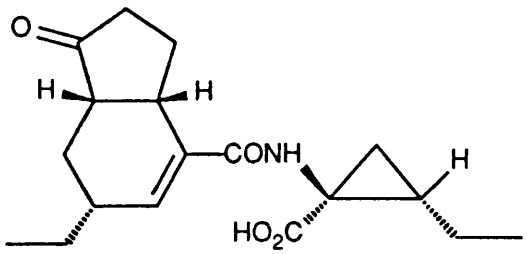
intermediates in the enzymatic and chemical decompositions respectively [200]. In these studies the required deutero compounds were prepared by alkylation of a glycine Schiff base with meso- and racemic dideuterodibromoethylenes; whilst the 2-methyl- and 2-ethyl-ACCs were also prepared [170] by condensing meso- and racemic 1,2-dibromopropanes with di-*t*-butyl malonate as described in Scheme 29. The racemic amino acid esters were then resolved and their absolute configurations determined [201].

The deamination of ACC by the microorganism *Pseudomonas sp.*, to generate α-ketobutyrate and ammonia, was reported in 1978 [202] and since then mechanistic studies of this conversion have appeared [203,204]. The preparation of optically active 2,2-dideutero-ACC, used in these studies [188-191], was achieved separately by two unique syntheses. One method uses a benzene ring as precursor of the required carboxyl function, and the other proceeding through a chiral epoxide [205].

Studies upon the stereochemistry of the enzymatic formation of ACC from S-adenosylmethionine in plants have also appeared [206-208]. The results indicate that inversion occurs at the γ-carbon atom of the methionine residue with direct intramolecular nucleophilic displacement of adenosyl methyl sulfide by a α-carbanion, to form the cyclopropane ring. Attempts to find alternate substrates and inhibitors of ACC synthetase from tomato plants showed that the enzyme accepts only very specific substrates [209]. Whilst in the rat brain ACC has been found to mimic the effects of glycine on the NMDA receptor [210].

(b)*Coronamic acid (***82***).*- Coronatine (**83**) is a toxin produced by *Pseudomonas corona-facience* which induces chlorosis in Italian ryegrass. It consists of a coronamic acid residue, *i.e.* (+)-(E)-2-ethyl-1-amino-1-cyclopropanecarboxylic acid, which has been N-acylated by coronafacic acid [211], a hydrinanonecarboxylic acid.

82



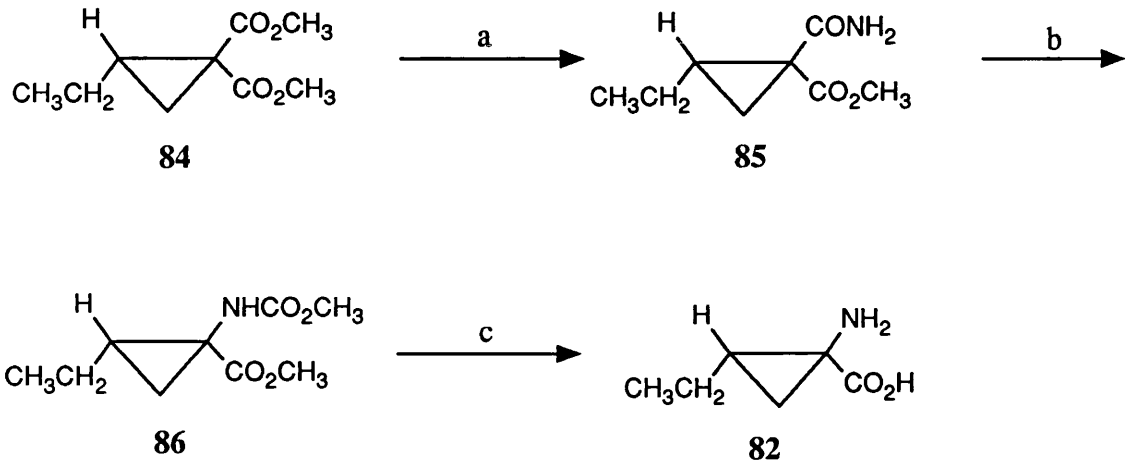
83

The first synthesis by Ichihara *et al* [212] of **82** (Scheme 31), began with the diester (**84**) prepared by dialkylation of dimethyl malonate with 1,4-dibromo-2-butene, followed by hydrogenation of the remaining vinyl group. Aminolysis of the less hindered ester function gave the amide (**85**), which was converted into the urethane (**86**) by Hofmann degradation. Hydrolysis of **86** gave racemic **82**, which was resolved as the brucine salt of the N-formyl derivative and by L-acylase hydrolysis of the N-acetyl derivative. Surprisingly, application of the sector rule [213] to this cyclopropane amino acid led to the conclusion that the (+)-form, obtained directly from the enzymatic hydrolysis, had the unlikely (1R, 2R)-configuration, *i.e.* unlikely if one assumes that the S-configuration of the α-carbon atom of a cyclopropane amino acid corresponds to that of a natural L-amino acid. This assignment was later revised [214] to the (1S, 2S)-configuration based on enzymatic oxidation experiments and X-ray crystallography. Thus indicating that the sector rule may not be applicable in the case of cyclopropane amino acids.

A synthesis of the optically active forms of allocoronamic acid was also reported by the same research group [215] in which the dimethylsulfoxonium methylide was used to cyclopropanate propylidenecyanoacetic acid ester, followed by resolution of the free amino acid *via* the quinine salt. A chiral synthesis of allocoronamic acid has been reported [216], but in poor yield. Later, a synthesis of racemic coronamic acid was

reported [171] *via* the addition of diazoethane to a dehydroalanine derivative (see Scheme 29), followed by pyrolysis and deblocking. Coronafacic acid has also been synthesized [217,218].

Scheme 31



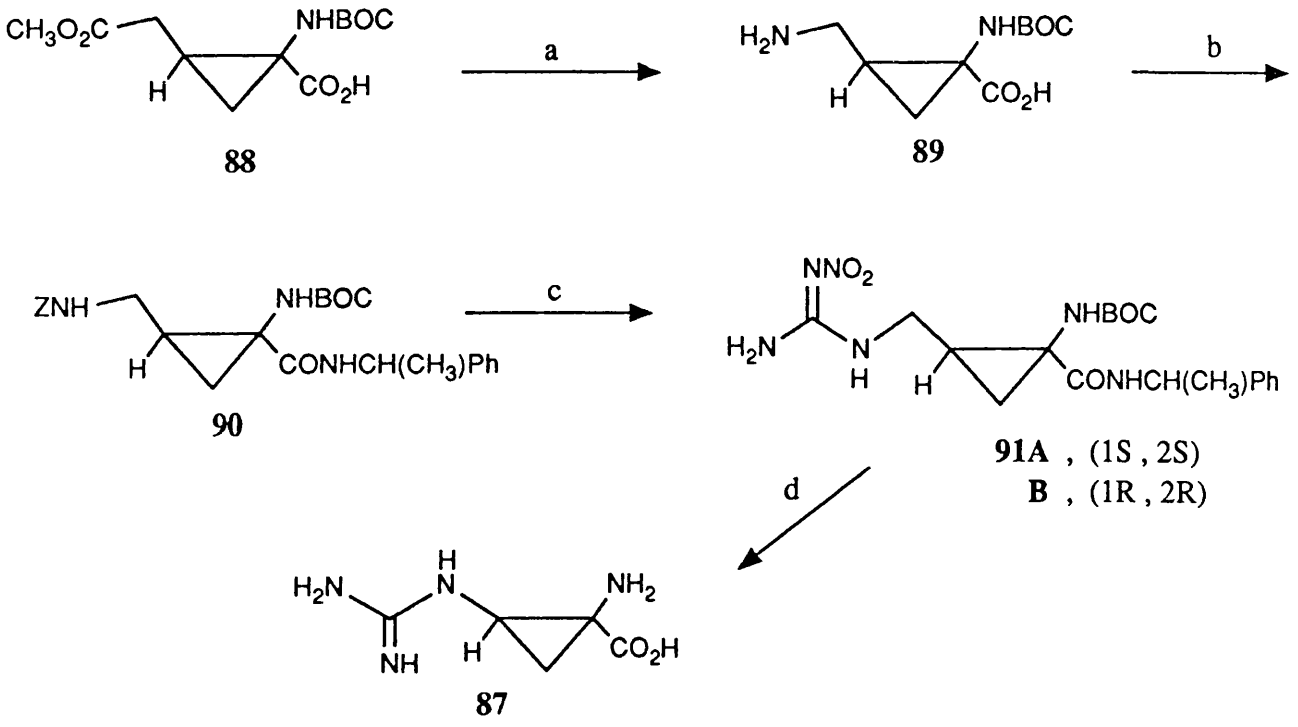
Reagents : (a) NH_3 , MeOH ; (b) Br_2 , NaOH ; (c) H_2O

Norcoronatine, a minor component of the phytotoxic fraction of *Pseudomonas syringae pv. glycinea* has been shown to contain norcoronamic acid, E-(1S, 2S)-2-methyl-1-aminocyclopropanecarboxylic acid [219] and a diastereoselective synthesis has been recently reported [220]. Studies of the biosynthesis [221], enzymatic deamination [222] and the bioactivity of a series of coronatine analogues [223] have also been published.

(c)*Carnosadine.*- In 1984, the isolation of a new 2,3-methanoamino acid, carnosadine (**87**) was reported [224] from a red alga, *Grateloupia carmosa.* This was followed by a report of its synthesis, by Wakamiya *et al* [225] (Scheme 32), which proceeded using the 2,3-methanoglutamic acid derivative (**88**), also synthetised for the first time. Conversion of the γ-ester function of **88** into an amino group by Hofmann degradation of the corresponding amide gave **89**, which was coupled with (R)-(+)-α-methyl

benzylamine, to give the amides (**90**), separable into the expected diastereomers. The γ-amino group was the deprotected, guanidated (3,5-dimethyl-1-nitroguanylpyrazole) and deblocked by hydrogenolysis, followed by hydrolysis to give **87**. The natural compound was shown to have the (1S, 2S) configuration.

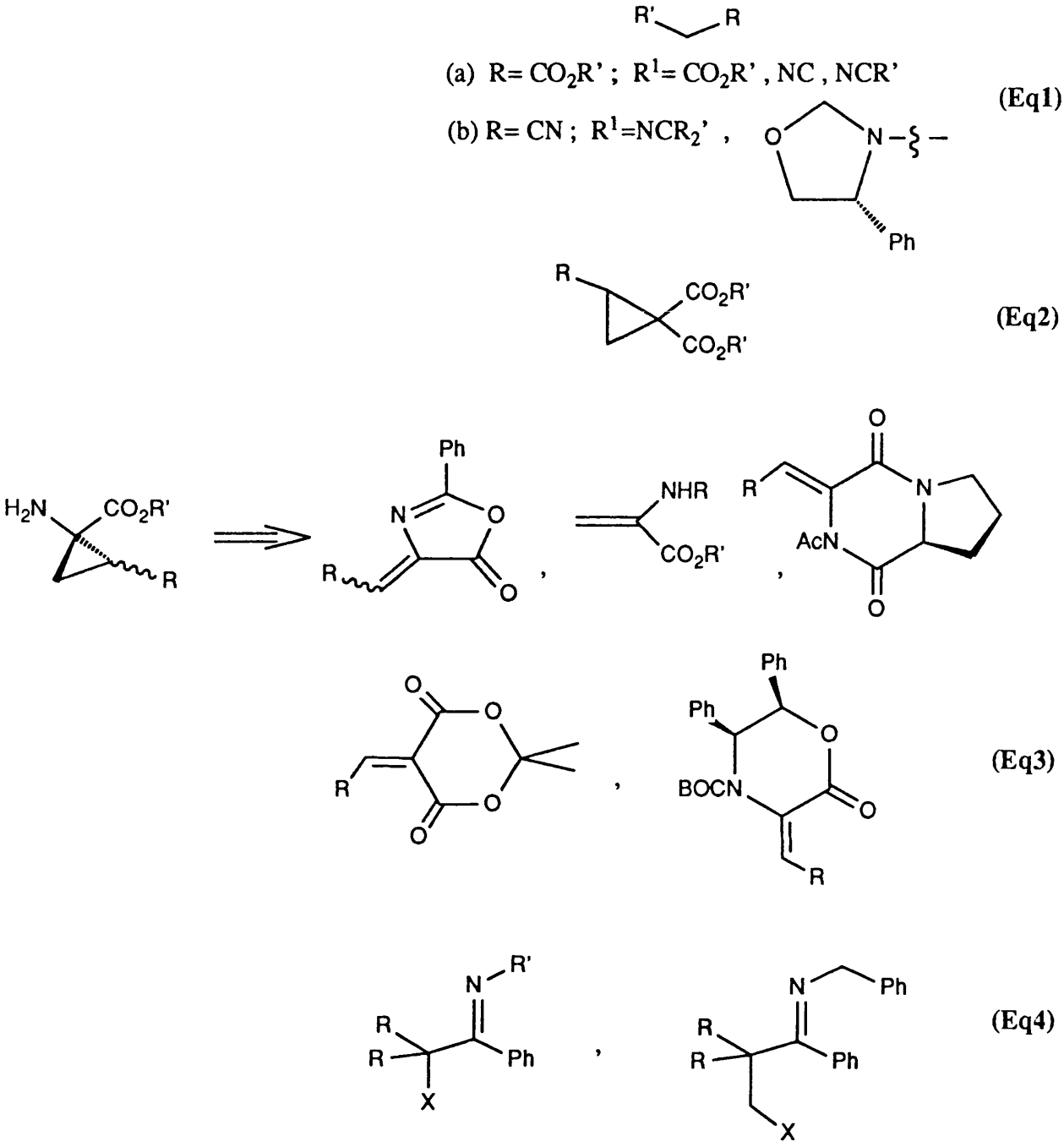
Scheme 32



Reagents : (a) i) NH_3 , MeOH , ii) Br_2 , NaOH ; (b) i) ZCl , NaOH , ii) (+) - $PhCH(CH_3)NH_2$, DCC , HOBT ; (c) i) H_2 / Pd , ii) DNG ; (d) i) H_2 / Pd , ii) 6M HCl .

Synthetic 2,3-Methanoamino Acids

Mono- and di-substituted ACCs provide a significant challenge to synthetic chemists due to the difficulty in controlling the relative and absolute stereochemistry around the cyclopropane ring. To date existing approaches to the synthesis of this class of amino acids include:-



(a) Tandem dialkylation of a glycine equivalent with a 1,2-dibromoalkane or a similar 1,2-disubstituted electrophile (**Eq. 1**).

(b) *Via* Curtius or Hofmann rearrangements of cyclopropane-1,1-dicarboxylates (**Eq.2**).

(c) Diazoalkane or dimethylsulfoxonium methylide addition to dehydroamino acid

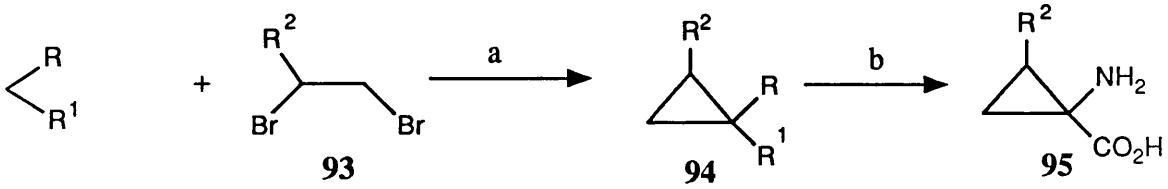
derivatives, followed by extrusion of N_2 gas or elimination of DMSO respectively (**Eq. 3**).

(d) Lewis acid activated ring opening of a substituted epoxide with a lithiated glycine equivalent followed by subsequent cyclisation (**Eq. 1**).

(e) Cyanide addition to α-chloroketimines or base-induced cyclisation of β-chloroimines (**Eq. 4**).

(a)*Via treatment of a glycine equivalent with a 1,2-disubstituted electrophile.*- In 1973, Schöllkopf reported [164] the synthesis of ACC *via* the subsequent treatment of an α-isocyanoester (**92A**) with base and the 1-substituted-1,2-dibromoethane (**93,** R^2=H) by acid hydrolysis of the intermediate α-isocyanocyclopropanecarboxylic ester (**94A**) (Scheme 33).

Scheme 33



92A , R= CO_2R , R^1= NC
B , R= CO_2R , R^1= CO_2R
Reagents : (a) base ; (b) H_3O^+

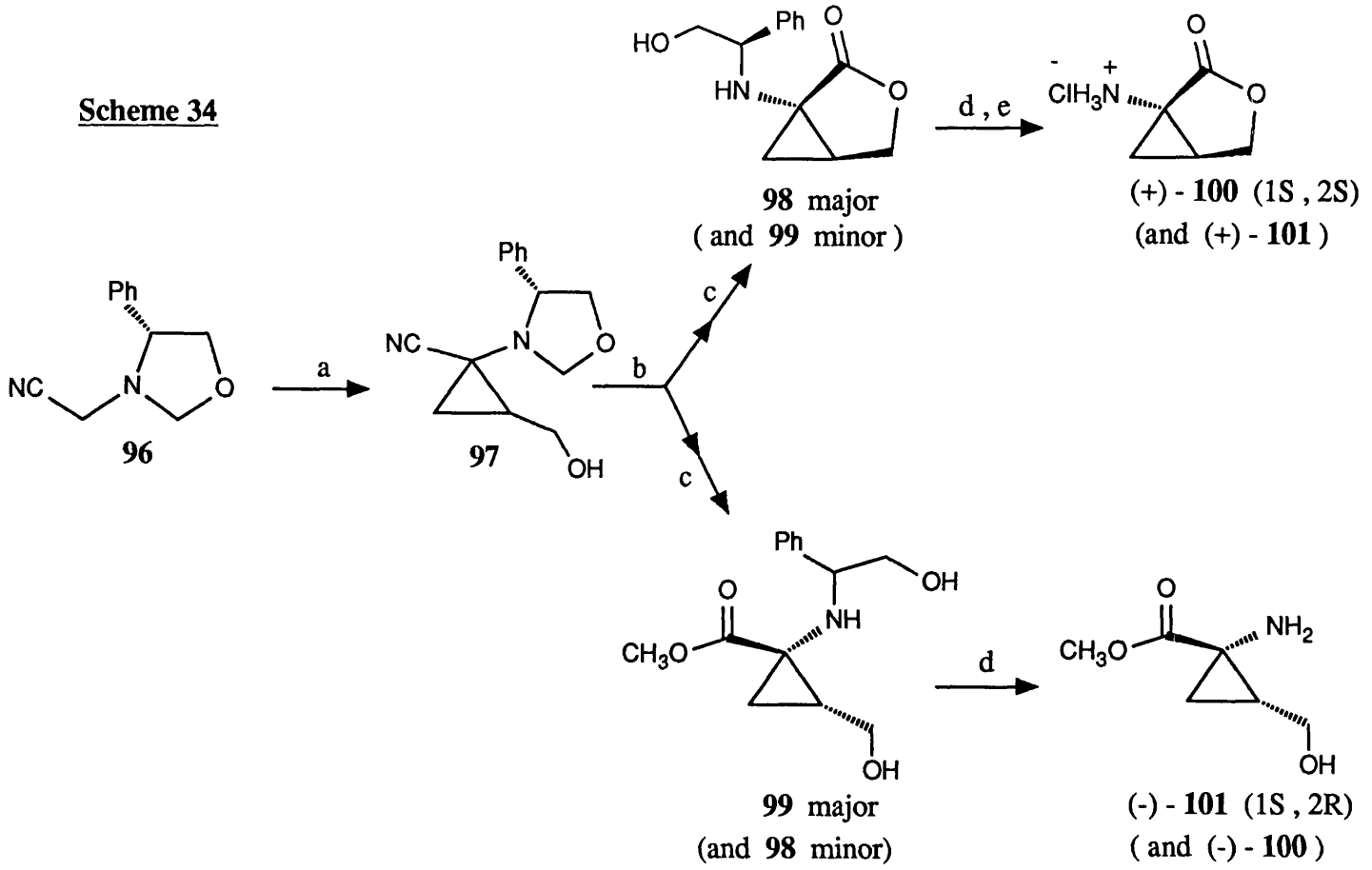
The methodology has since been repeated by Pirrung *et al* [226] who prepared a 2-cyclopropyl derivative (**95A**) as a mixture of diastereomers (>7:1). Alternatively, Baldwin [227] reported the cyclisation of the ester (**92B**) to the cyclopropane and then selectively hydrolysed the less hindered ester function with base. After acidification, the carboxylic acid obtained was activated to the mixed anhydride with ethyl chloroformate, treated with sodium azide at 0°C, refluxed in toluene and hydrolysed with hydrochloric acid to afford the (E)-2-alkyl-2,3-methanoamino acids in good yield.

The preparation of allocoronamic acid has been achieved by using epihalohydrins [216,228,229] or by other α-substituted epoxides [230]. The glycine equivalent (**96**) has been shown [228] to dialkylate at the aminonitrile function *via* treatment with strong base and epibromohydrin, to produce all four possible cyclopropyl diastereomers (**97**) through cyclisation, two of which predominated (Scheme 34).

Careful chromatography of **97** allowed the separation of approximate equal quantities of two pairs of components, each of which contained one major and one minor product, in a ratio of approximately 6:1 for both pairs. It transpired that in the first pair the major component had a *cis*-relative configuration of the nitrile and hydroxymethyl substituent, whilst in the second pair the major component had a *trans* configuration. The difference in substituent geometry within each pair was exploited and allowed further separation *via* sequential treatment with aqueous base, acid and thionyl chloride/methanol mix to effect esterification. The cyclopropane possessing the *cis* configuration underwent exclusive γ-lactone formation to give **98**, while those with the *trans* configuration yielded the monocyclic methyl esters (**99**). Separation of the *cis* and *trans* derivatives was achieved, once the chiral auxiliary had been removed, by hydrogenolysis to give the primary amino compounds(+)-(**100**) and (-)-(**101**) respectively.

(*b*)*Via Curtius or Hofmann rearrangements of cyclopropane-1,1-dicarboxylates.*- The Curtius and Hofmann rearrangements [231,232] are invaluable procedures for the conversion of azides (**103**) and primary amides (**104**) through to isonitriles. In the case of the Hofmann rearrangement, the isonitrile formed is usually hydrolysed under the reaction conditions to afford the primary amine. Whereas the isonitrile can be isolated in the Curtius procedure, or subsequently transformed into amines, carbamates, *etc.* upon treatment with the appropriate reagent : water, alcohol, *etc.*

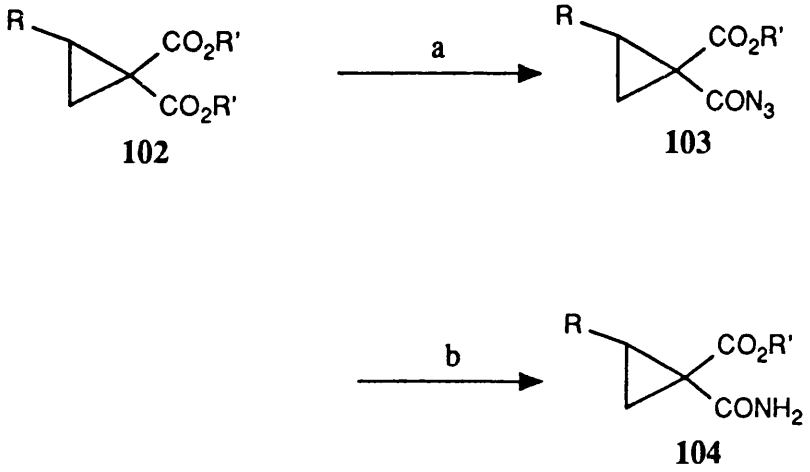
Scheme 34



Reagents : (a) i) LDA , HMPT , 2.1 eq. , THF , -70^0 C , 45 min.. , ii) epibromohydrin , THF, -70^{0}C , 1h . ; (b) Chromatography (silica , 40:60 EtOAc : hexane) ; (c) i) NaOH , Δ , 20 h , ii) H_3O^+ , pH 2-3 , room temp. , iii) $SOCl_2$, MeOH , Δ , 3 h ; (d) i) 3 atm. H_2 , 10 % Pd / C , MeOH , 20h , ii) Chromatography (silica ,10 : 90 MeOH : EtOAc) ; (e) HCl , EtOH .

The initial cyclopropane-1,1-dicarboxylates (**102**) can be readily transformed into the primary amides or azides *via* treatment with methanolic ammonia or potassium hydroxide/sodium azide, respectively. This takes place through the selective hydrolysis of the less hindered ester function (Scheme 35).

Scheme 35

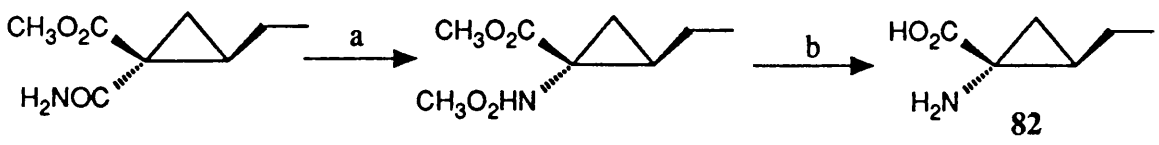


102 **103** **104**

Reagents : (a) KOH , NaN_3 ; (b) NH_3 , MeOH

These methods have been frequently used [212,227,233,234] in the preparation of 2,3-methanoamino acids providing stereochemical retention and good yields of the desired products. For example, Ichihara *et al* [212] used a Hofmann rearrangement in their preparation of coronamic acid (**82**)(Scheme 36).

Scheme 36

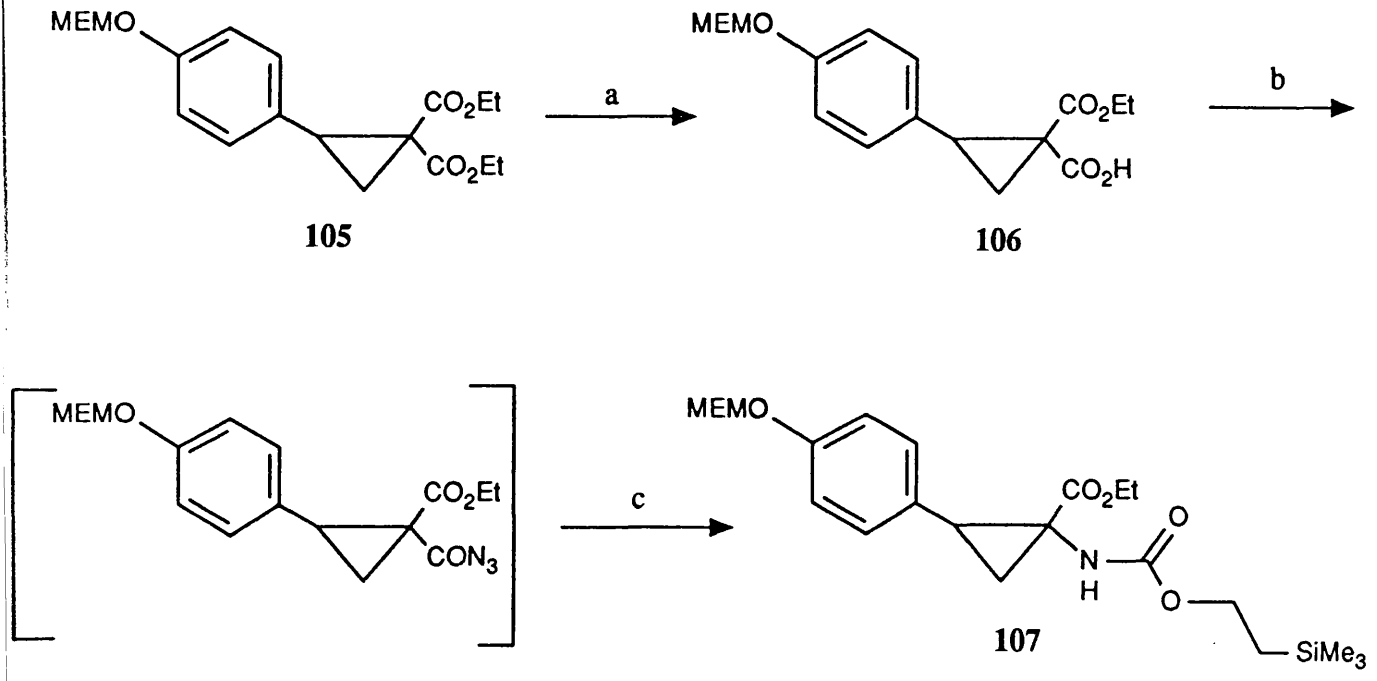


82

Reagents : (a) Br_2 , NaOH , MeOH ; (b) H_3O^+

Whereas, in the preparation of 2,3-methanotyrosine, Stammer *et al* [233] have transformed the monoester (**106**), (prepared through selective hydrolysis of **105** with potassium hydroxide), to the silyl carbamate (**107**) *via* a Curtius rearrangement. This involved the treatment of **106** with diphenylphosphazidate (DPPA) with reflux, followed by esterification of the resulting isocyanate with 2-(trimethylsilyl)ethanol, to afford **107** in a reasonable yield (53%) (Scheme 37).

Scheme 37



Reagents : (a) aq. KOH ; (b) DPPA , Et_3N , $PhCH_3$; (c) $(CH_3)_3SiCH_2CH_2OH$, Δ .

(c)*Diazoalkane or dimethylsulfoxonium methylide addition to dehydroamino acids.*-

The substrates used in these methylene insertions fall into five types, namely:-

(i) Oxazolone derivatives

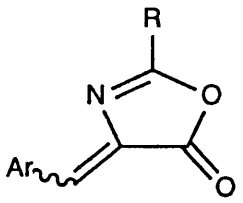
(ii) Dehydroamino acid derivatives

(iii) Piperazin-2,5-dione derivatives

(iv) Meldrum's acid derivatives

(v) 1,4-Oxazin-2-one derivatives

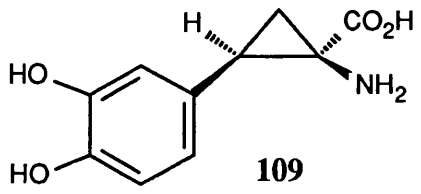
(i)*Oxazolone derivatives.*- Oxazolone derivatives of the structure (**108**) have been prepared since the 1930's.



108

They are generally prepared by the Erlenmeyer-Ploch reaction [200] *via* the condensation of aldehydes or ketones with oxazolones (old name azlactones) (See section 1.3). The stable isomer in all cases has the (Z)-configuration in which the aryl group and the carboxyl function have the *trans* orientation.

Initially the isolation of 2,3-methanoamino acids, as indeed for dehydroamino acids, was hampered by the inability to remove the acyl group from the amino function of the newly assembled amino acid; as was found by Stammer [235] in the attempted preparation of 2,3-methano-3′,4′-dihydroxyphenylalanine (**109**) as a potential 3′,4′-dihydroxyphenylalanine (DOPA) decarboxylase inhibitor.

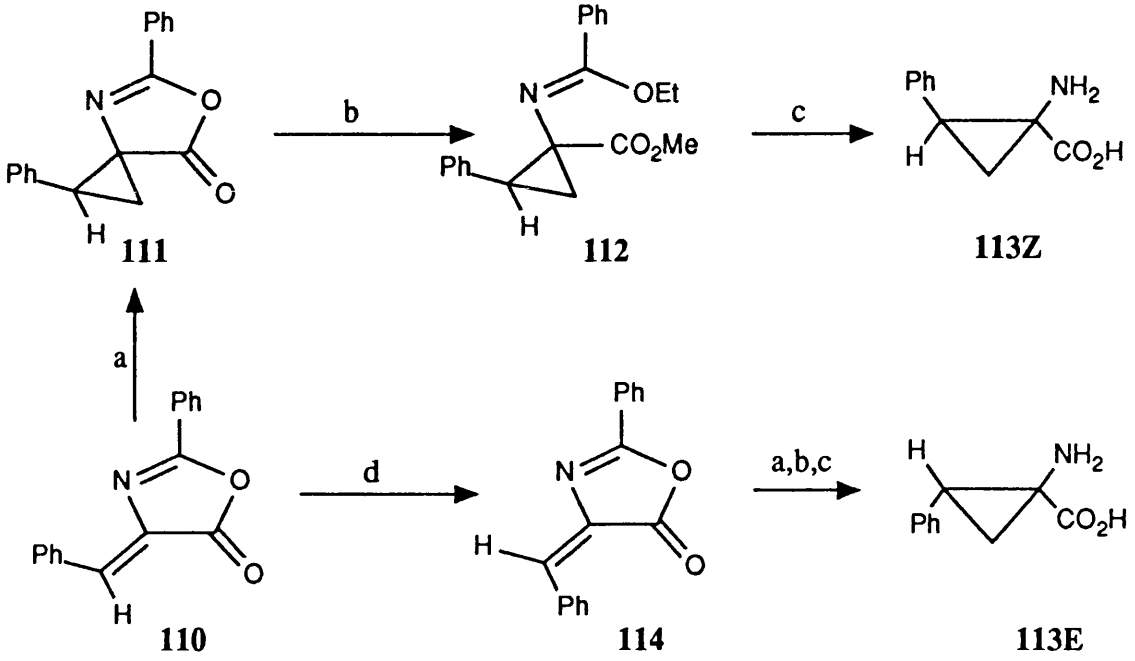


109

This was overcome by Stammer [173] in the first synthesis of the (E)- and (Z)- isomers of racemic 2,3-methanophenylalanine (**113**) from the oxazolone (**110**) *via* methylation of the N-benzoyl derivative (**112**) to the imidate salt, followed by mild hydrolysis

(Scheme 38).

Scheme 38



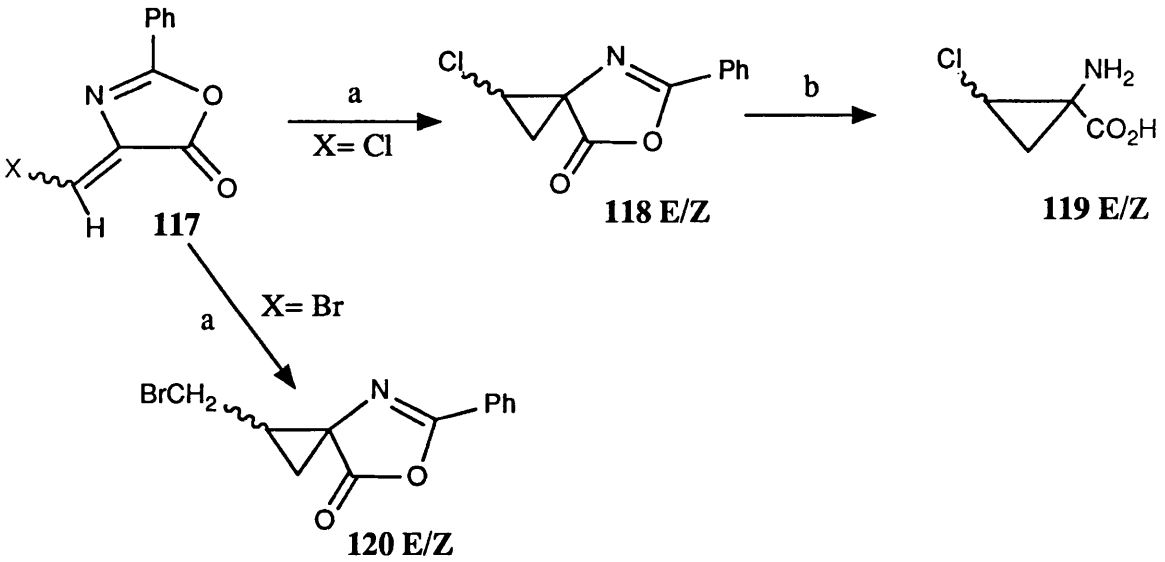
Reagents : (a) CH_2N_2 ; (b) i) MeOH , DMAP , ii) $Et_3O^+BF_4^-$; (c) i) 1N HCl , room temp. , ii) NaOH , iii) AcOH ; (d) HBr .

The instability of the cyclopropane ring to strong acid hydrolysis and to hydrogenolysis [235] was circumvented during the synthesis of some twenty-four aromatic ring-substituted 3-aryl-2,3-methanoamino acids by the use of the sulfur-containing heterocycles, 4-arylidene-2-benzylthio-5(4H)-thiazolones [236], which are cleavable to the amino acids by strong aqueous base. A number of these 2,3-methanophenylalanines prepared by this method were shown to be reversible time-dependent inhibitors of both DOPA decarboxylase [237] and tyrosine aminotransferase [238]. Further work concerning the reaction of diazomethane with oxazolones [239-240], thiazolones [241] and (E)- and (Z)-2-acylaminocinnamates [242] have been reported.

Reports have been made of the preparation of the cyclopropane analogues of

(Z)-2,3-methanothyronine, its 3′,5′-dibromine derivative [243] and (Z)-2,3-methanohistidine [244], through treatment of the corresponding oxazolones with diazomethane. These spirooxazolones were reportedly converted into the desired 2,3-methanoamino acids by acid catalysed hydrolysis without apparent decomposition. The thyronine derivatives showed little thyroxine-like activity whilst the histidine analogue had a weak inhibitory effect on histidine carboxylase.Both 2,3-methanohomoserine (**115**) and 2,3-methanomethionine (**116**) were prepared in the process of investigating the cyclopropanation of heteromethylidine-oxazolones (**117**) [245] (Schemes 39 and 40).

Scheme 39

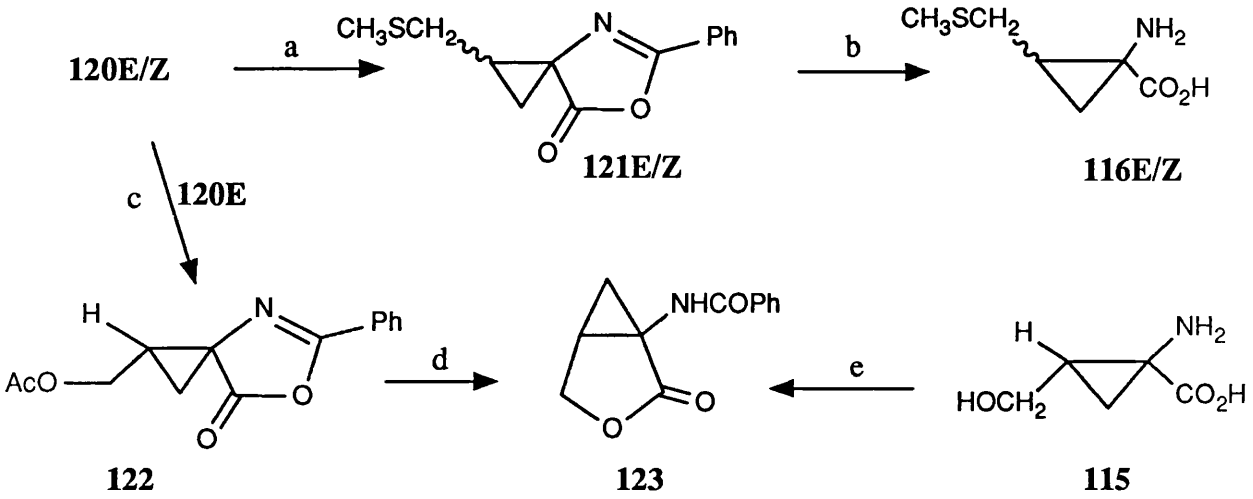


Reagents : (a) CH_2N_2 ; (b) HCl , H_2O .

The treatment of **117**, X=Cl, with diazomethane yielded a 1:1 mixture of (E)- and (Z)-isomers of the cyclopropanes (**118**), which in turn were taken through to the (E)- and (Z)-diastereomers of 2-chloro-1-aminocyclopropane-carboxylic acid (**119 E/Z**). The bromo derivatives of **117**, X=Br, however, gave five compounds upon methylene insertion, separable by chromatography, of which the insertion products predominated (**120 E/Z**). These (E)- and (Z)-isomers of **120** were separately converted into the

methylthio derivatives (**121 E/Z**) and then hydrolysed to give the diasteromers of 2,3-methanomethionine (**116,** Scheme 40) [246].

Scheme 40



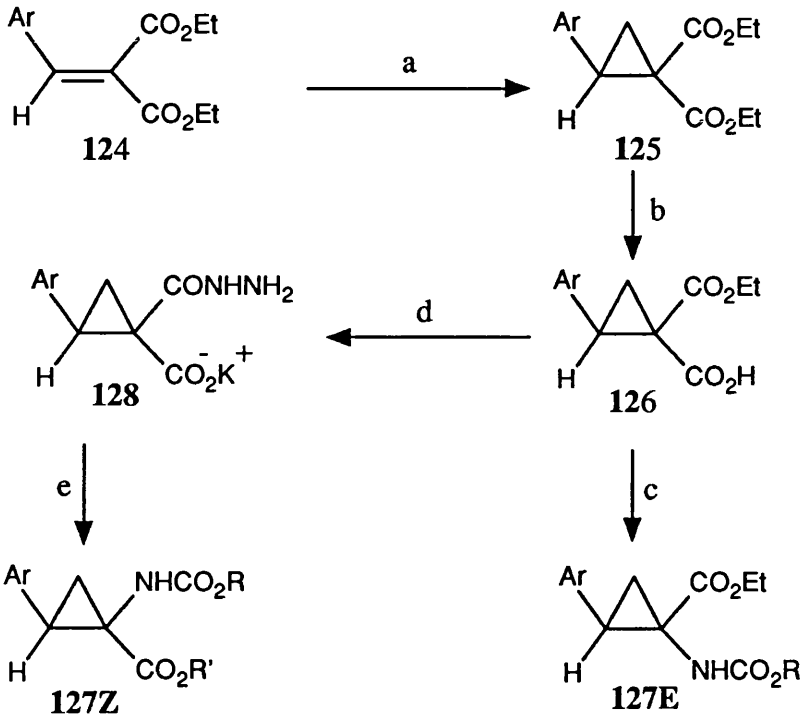
Reagents : (a) CH_3SNa , CH_3OH ; (b) HCl , H_2O ; (c) KOAc , H_2O ; (d) HCl ;
(e) i) PhCOCl , NaOH , ii) HCl .

In order to prove the configurations of these compounds, the presumed (E)-isomer (by NMR) of **120** was converted into the lactone (**123**), prepared also from (E)-2,3-methanohomoserine (**115**). Comparison of this with an independent synthesis of **115**, verified that it had the (E)-configuration.

(ii)*Dehydroamino acid derivatives*.- The malonate method, first reported in a patent [247], has been used for the synthesis of aromatic 2,3-methanoamino acids. This is the cyclopropanation of an arylidene malonic acid diester with a dimethylsulfoxonium methylide [248], followed by the introduction of the amino group *via* an azide and consequent Curtius rearrangement. This method (Scheme 41) has two advantages: firstly, either diastereomer of the target amino acid can be synthesized at will; and secondly, diazo compounds are not used. This method is, however, longer than the oxazolone process since one of the ester functions must be converted into an amino

group.

Scheme 41

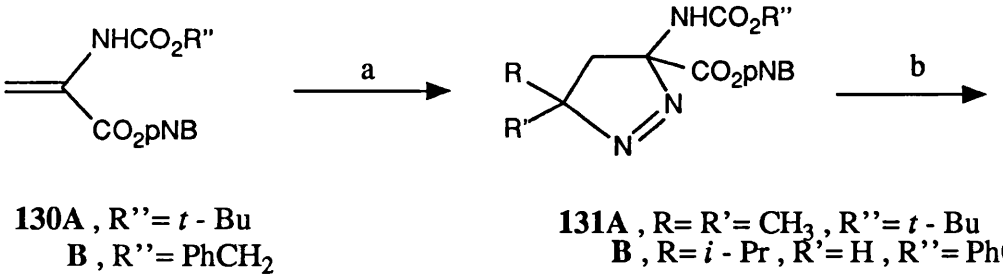


Reagents : (a) $(CH_3)_3S^+(O)CH_2^-$; (b) NaOH , H_2O ; (c) $(PhO)_2PON_3$,ROH ; (d) i) $KHCO_3$, EtOH , H_2O , ii) NH_2NH_2 , H_2O , EtOH , Δ ; (e) i) 1M H_2SO_4 , $NaNO_2$, Et_2O , ii) CH_2N_2 , Et_2O , iii) R'OH , $PhCH_3$, Δ

Both diastereomers of 2,3-methanotyrosine have recently been synthesized by this method [233]. The cyclopropane (**125**) is easily saponified to give the less sterically hindered acid (**126**) which is rearranged to the isocyanate with diphenylphosphoryl azide. Conversion to the urethane (**127E**) was achieved *in situ* by isocyanate alcoholysis, and the final deblocking steps depend upon the alcohol used. The (Z)-isomer (**127Z**) was obtained from **126** *via* hydrazinolysis of the ester function followed by nitrosation, rearrangement and alcoholysis as before. Resolution of the racemic amino acids obtained by this method is necessary if the optical isomers are desired.

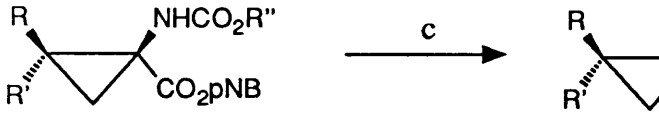
In the case of aliphatic 2,3-methanoamino acids, several different approaches have been used. Racemic 2,3-methanovaline (**129A**) and both (Z)- and (E)-2,3-methanoleucines (**129B** and **129C**) were prepared by the additon of a substituted diazomethane to a dehydroalanine derivative (**130**) [249] (Scheme 42).

Scheme 42



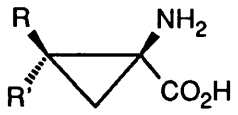
130A , R''= *t* - Bu
B , R''= $PhCH_2$

131A , R= R'= CH_3 , R''= *t* - Bu
B , R= *i* - Pr , R'= H , R''= $PhCH_2$



132A , R= R'= CH_3 , R''= *t* - Bu
B , R= *i* - Pr , R'= H , R''= $PhCH_2$
C , R= H , R'= *i* - Pr , R''= $PhCH_2$

133A , R= R'= CH_3 , R''= *t* - Bu
B , *i* - Pr , R'= H , R''= $PhCH_2$
C , R= H , R'= *i* - Pr, R''= $PhCH_2$



129A , R= R'= CH_3
B , R= *i* - Pr , R'= H
C , R= H , R'= *i* - Pr

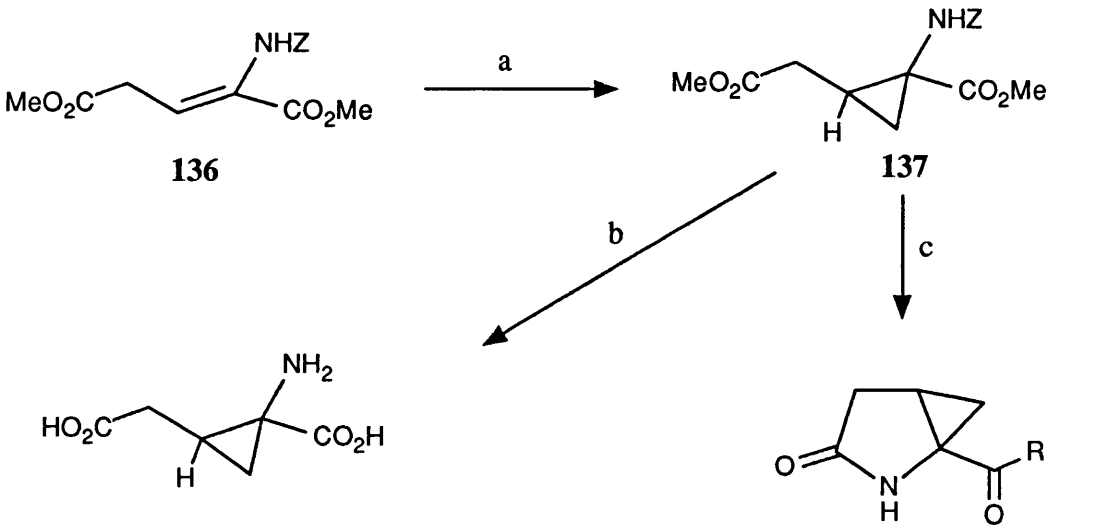
Reagents : (a) $RR'CN_2$; (b) Δ or hν ; (c) NaOH , H_2O ; (d) HCl or H_2 , 5% Pd / C .

Photolylic conversion, which is preferable to pyrolysis, of the pyrazolines (**131**) to the cyclopropanes (**132**), followed by standard deblocking procedures, allowed the final

isolation of the amino acids. The aliphatic 2,3-methanoamino acids are stable to both hot aqueous acid and hydrogenolysis (5% Pd/C) at atmospheric pressure. Thus, compound **129A** was prepared on the removal of the N-t-butyl group by strong hydrolysis of 1-N-t-butyl-1-cyano-2,2-dimethylcyclopropane (**133A**).

The synthesis of racemic (Z)-2,3-methanoglutamic acid (**134**) reported recently [225], has allowed the preparation of racemic 2,3-methanopyroglutamic acid to be achieved (**135B**) (Scheme 43) [250,251].

Scheme 43

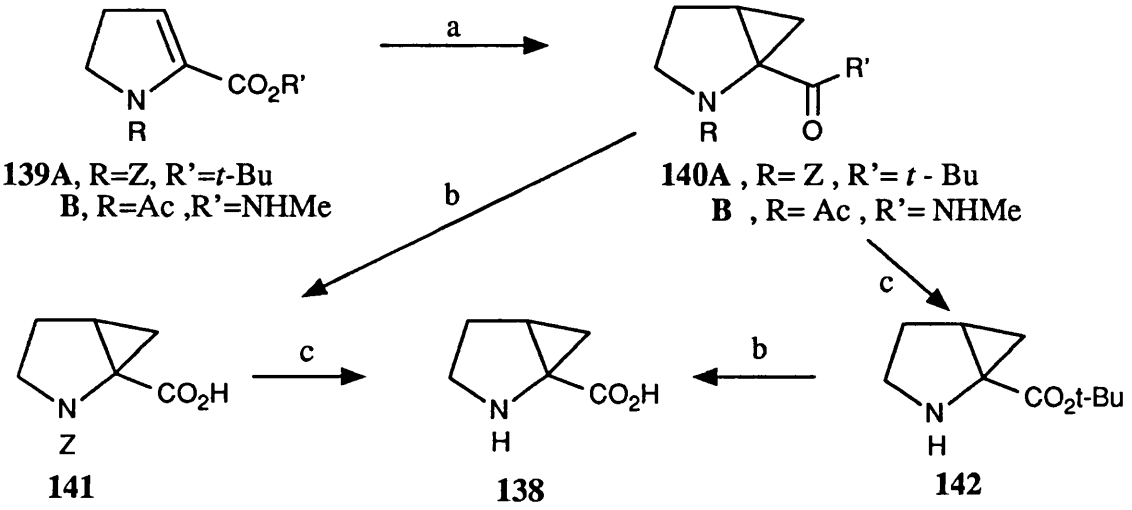


Reagents : (a) i)CH_2N_2 , ii) hν ; (b) 6M HCl , Δ ; (c) i) H_2 , Pd /C , ii) Δ , iii) NaOH , H_2O .

Cyclopropanation of a (Z)-dehydroglutamic acid derivative (**136**), prepared by the reaction of benzyloxycarbonylamide with 2-ketoglutaric ester, was accomplished by the addition of diazomethane to **136**, followed by photolysis of the resulting pyrazoline. Acidic hydrolysis of the cyclopropane **137** gave the free amino acid in good yield. Hydrogenolysis of the benzyloxycarbonyl group of **137**, followed by ring closure in

boiling *sec*-butyl alcohol, gave the 2,3-methanopyroglutamic acid ester (**135A**), which was readily hydrolysed to the desired 2,3-methanopyroglutamic acid (**135B**). An X-ray structure of the ester (**135A**) and an NMR study of the conformation of N-methylamide (**135C**) have also appeared [250,251]. The β-naphthalimide (**135D**) was found to be stable to enzymatic hydrolysis by pyroglutamate aminopeptidase *in vitro* [250].

Scheme 44



139A, R=Z, R'=*t*-Bu
B, R=Ac ,R'=NHMe

140A , R= Z , R'= *t* - Bu
B , R= Ac , R'= NHMe

141

Z= $PhCH_2OCO$-

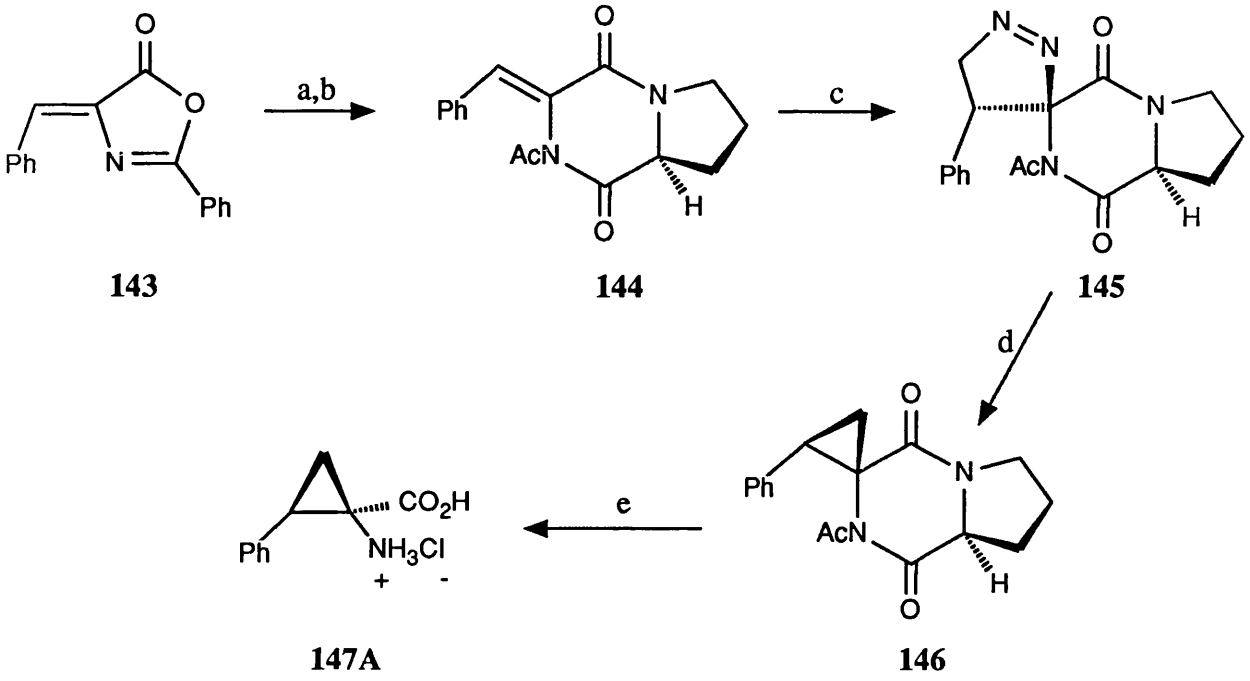
138

142

Reagents : (a) i) CH_2N_2 , ii) hv ; (b) CF_3CO_2H ; (c) H_2 / Pd .

The synthesis of racemic 2,3-methanoproline (**138**) has been accomplished recently [252] by diazomethane cyclopropanation of the 2,3-dehydroproline derivative (**139**) (Scheme 44), followed by deblocking. The racemic form (**138**) was found to be a weak inhibitor of the methylene forming enzyme in cucumber cotyledons and squash seeds. NMR studies performed on the N-acetyl-N-methylamide (**140B**) indicated that the 2,3-methano compound showed a slightly greater preference for the *cis* amide bond at the ring nitrogen atom than the same derivatives of proline. An X-ray crystal structure on **140B** indicated a small ψ angle (7°) as compared to the same derivative of proline (16°), but an almost identical ϕ angle (76°).

Scheme 45



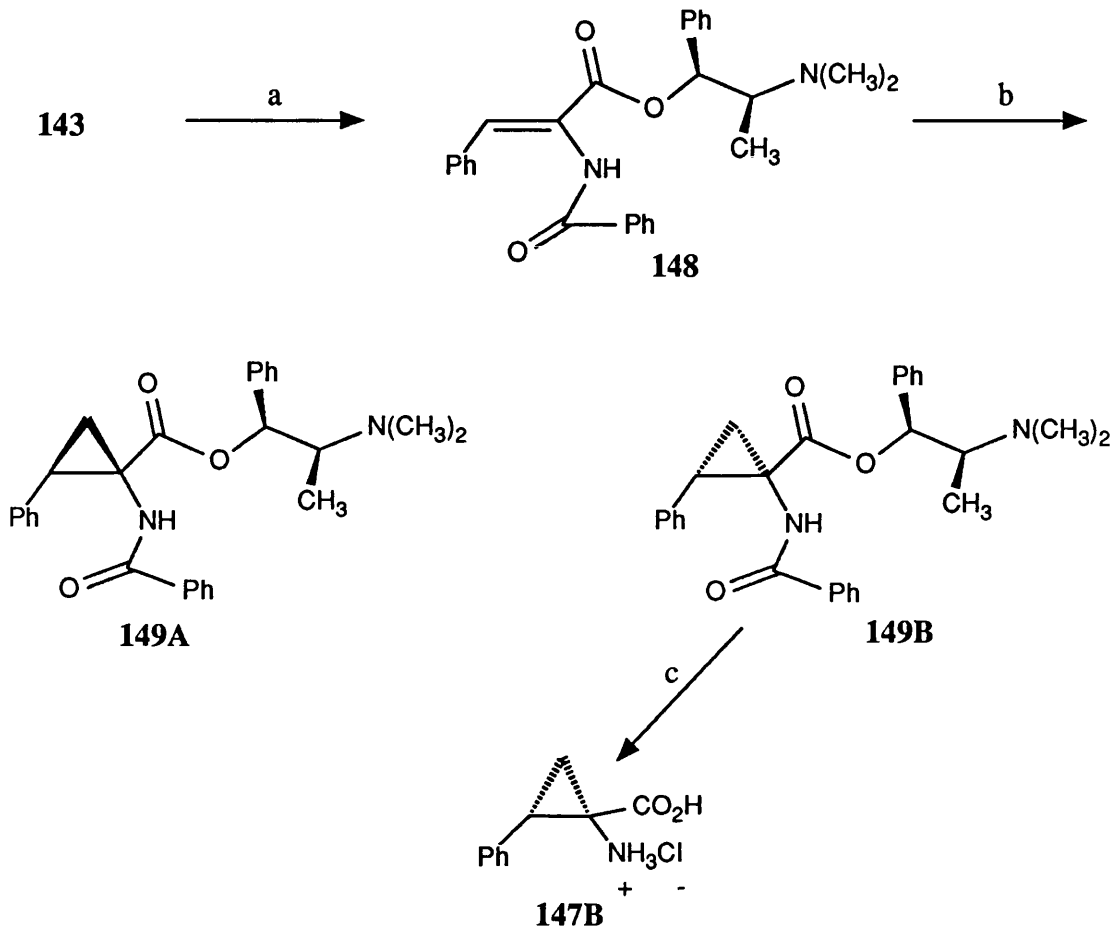
143 **144** **145**

147A **146**

Reagents : (a) NaOH , L-Proline , H_2O , acetone ; (b) Ac_2O ; (c) CH_2N_2 , benzene ; (d) hv , benzene ; (e) 6N HCl , AcOH .

(iii)*Piperazin-2,5-dione derivatives.*- A recent chiral synthesis [253] of (Z)-2,3-methanophenylalanine was reported in which the oxazolone (**143**) was converted into an optically active piperazin-2,5-dione (**144**) with L-proline, and to a chiral ester (**148**) with (-)-N-methylephredine, before diazomethane cyclopropanation was employed (Schemes 45 and 46 respectively); the first study (Scheme 45) producing almost a single diasteromer (>95% de) of the pyrazoline (**145**) in good yield (70%).

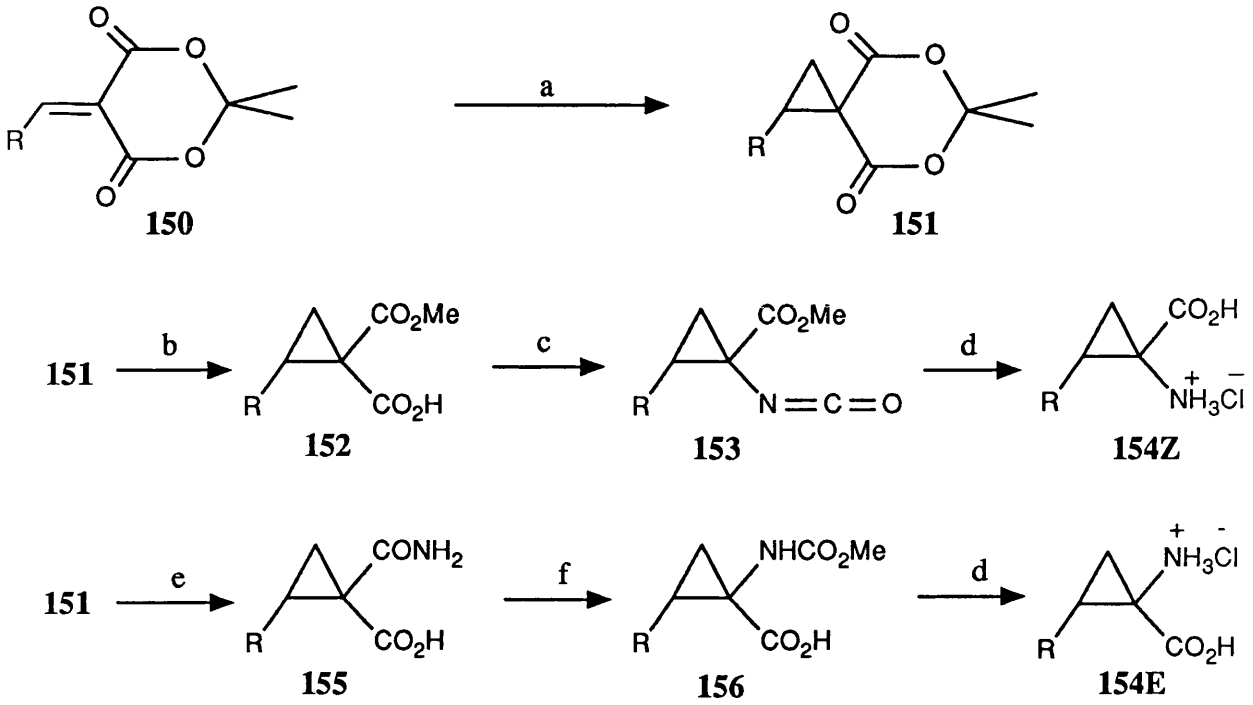
Scheme 46



Reagents : (a) (-) -N- methylephredrine , NaH , THF ; (b) CH_2N_2 , benzene ; (c) 6N HCl , dioxane .

(iv)*Meldrum's acid derivative.*- The reaction of 5-arylidene Meldrum's acid derivatives (**150**) with dimethylsulfoxonium methylide gave the racemic spirocyclopropanes (**151**) in good yield [254]. Treatment of **151** with sodium methoxide, followed by Curtius-type treatment, yielded the isocyanates (**153**), which on hydrolysis gave the corresponding (Z)-2,3-methanoamino acid salts (**154Z**). Whilst the addition of ammonium hydroxide to **151** gave the amide salt (**155**), which under Hofmann rearrangement conditions and subsequent hydrolysis of the generated carbamate (**156**), gave the (E)-2,3-methanoamino aicd salts (**154E**) (Scheme 47).

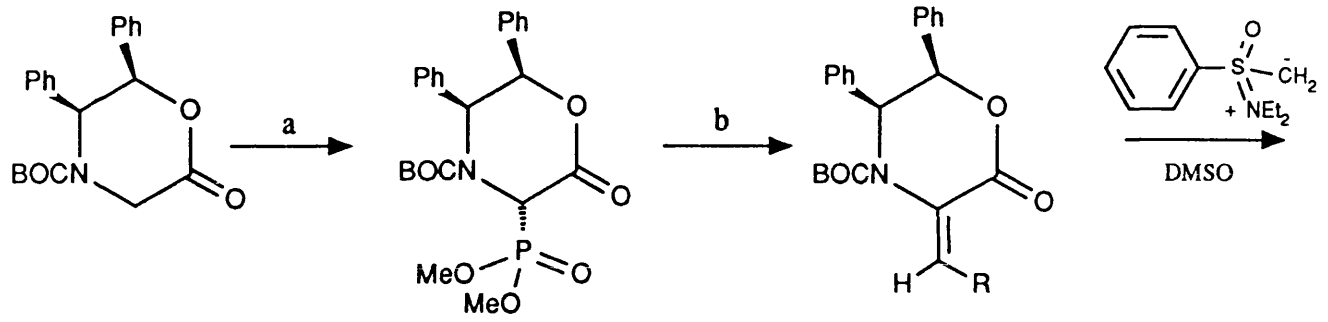
Scheme 47



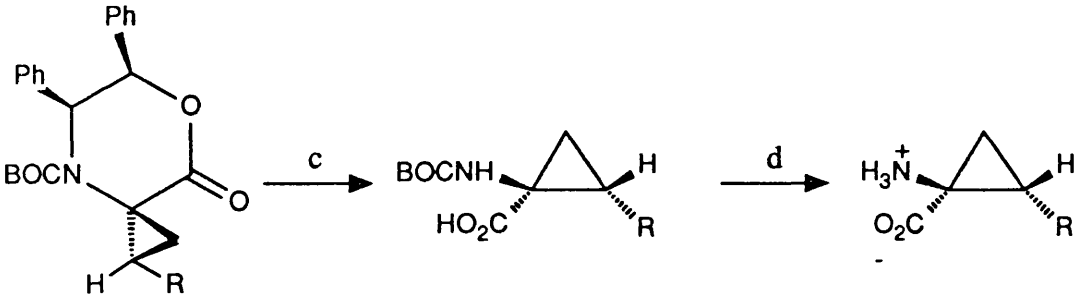
Reagents : (a) $CH_2^-S^+O(CH_3)_2$; (b) NaOMe , MeOH ; (c) i) EtOCOCl , Et_3N , acetone , ii) NaN_3 , H_2O ; (d) HCl ; (e) NH_4OH ; (f) i) Br_2 , MeOH , ii) NaOMe , MeOH .

(v)*1,4-Oxazin-2-one derivatives*.- Optically active 1,4-oxazin-2-one derivatives (**158**) have been efficiently condensed with various aldehydes *via* the Emmons-Horner-Wadsworth procedure [255] to provide α,β-dehydrolactone adducts (**159**) in good yield [256]. It was found that the adducts (**159**) were cyclopropanated with the ylide of racemic {[(diethylamino)methyl]phenyl}oxosulphinium tetrafluroborate to furnish in high chemical and optical yields (>99% ee) the desired (E)-cyclopropanes (**160**). Final deblocking of **160** gave the requisite 2,3-methanoamino acids *via* dissolving-metal reduction (Scheme 48).

Scheme 48



157 **158** **159**

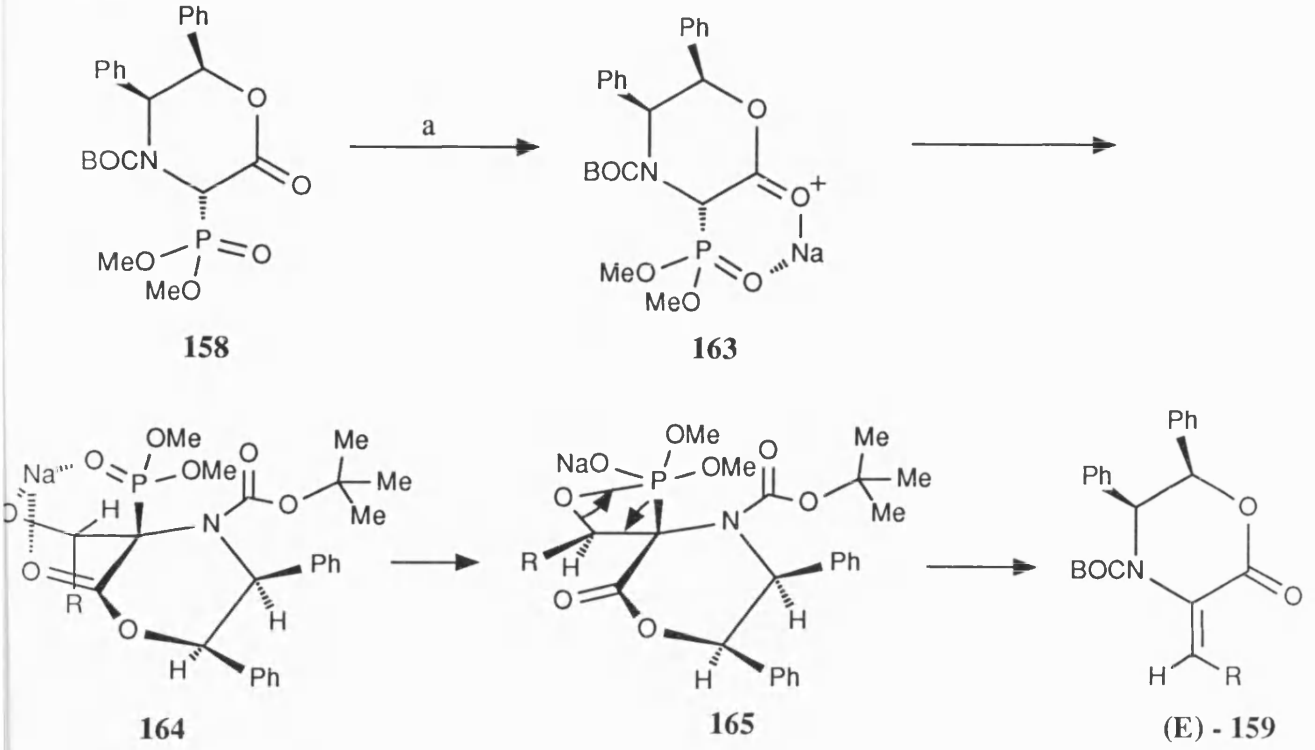


160 **161** **162**

Reagents : (a) i) NBS , CCl_4 , Δ , ii) $(MeO)_3P$, THF ; (b) i) NaH , THF , ii) RCHO ; (c) Li , NH_3 , THF , EtOH ; (d) i) HCl , MeOH , ii) propylene oxide , EtOH , Δ .,

X-ray studies upon **159** and the N-*p*-bromobenzoyl derivative of **160** had indicated that the alkene geometry was preserved and that the sulfoxime ylide attack on the lactone (**159**) proceeded from the top (β) face of the double bond. The unusual (E)-selectivity of the olefination and the complete absence of the (Z)-alkene (**159Z**) was thought to be due to the steric interaction between the aldehyde R group and the bulky BOC protecting group being unfavoured in the betaine transition state (see **164**, Scheme 49).

Scheme 49

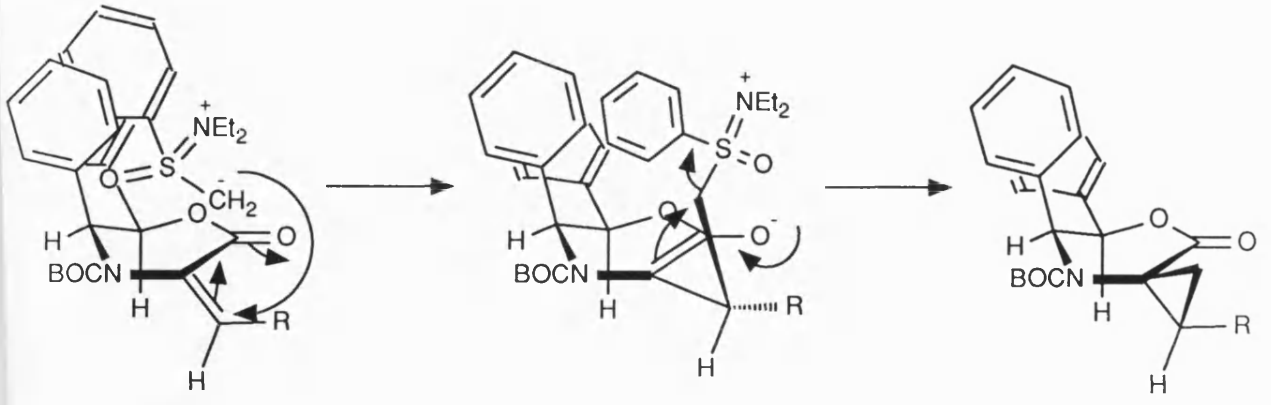


158 **163** **164** **165** **(E) - 159**

Reagents : (a) NaH , RCHO .

Also, the high degree of facial selectivity was thought to be due to π stacking interactions of the two phenyl rings of the oxazinone with the phenyl ring of the ylide, thus introducing the methylide from the β-face of the double bond (Scheme 50).

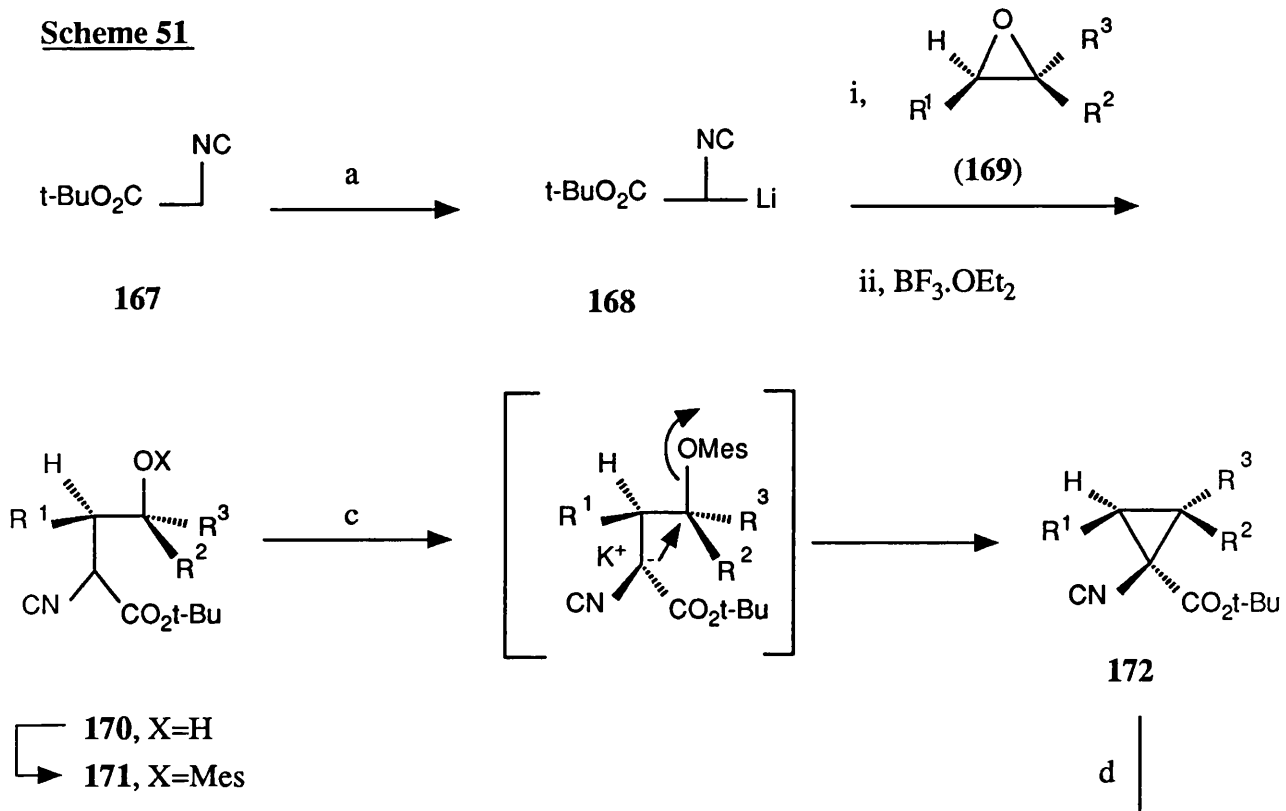
Scheme 50



159 **166** **160**

Scheme 51

Scheme 51



Reagents : (a) BuLi , THF ;

(b) MsCl , Et_3N , DCM ;

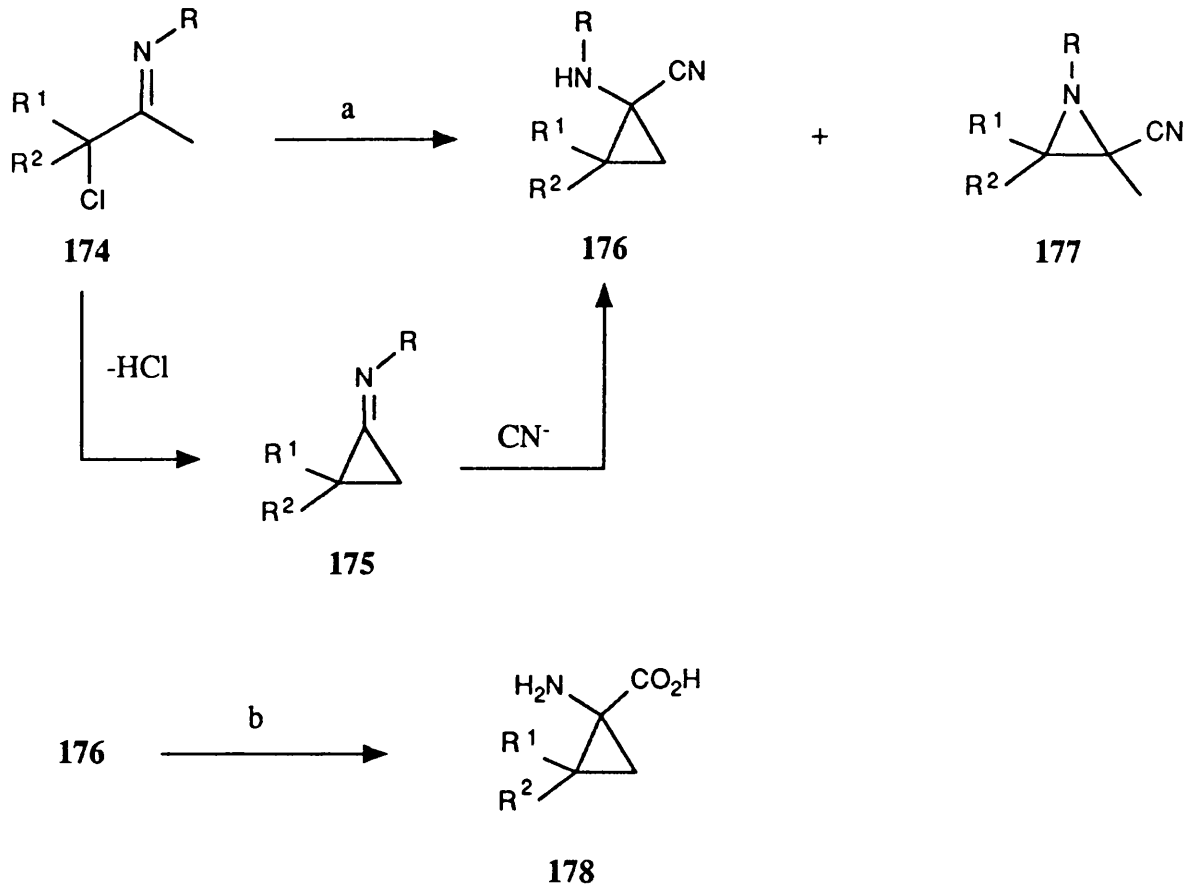
(c) *t*-BuOK , THF.

(d) i) H_3O^+,ii) neutralise

(d)*Via Lewis acid activated ring opening of a substituted epoxide with a lithiated glycine equivalent.*- Lithiated *t*-butyl isocyanates (**168**) have been shown [257] to react with epoxides (**169**) in the presence of boron trifluoride ethers to give exclusively the *t*-butyl-4-hydroxy-2-isocyanoalkanoates (**170**) (Scheme 51). The substitution took place with inversion of configuration of the least hindered carbon centre of the epoxide. The γ-hydroxy ester (**170**), upon mesylation, underwent base induced cyclisation giving the 1-isocyano-1-cyclopropane carboxylates (**172**) with *trans* configuration (CO_2t-Bu with respect to R^1 and R^2) with high diastereoselectivity (>95:5 *trans:cis*) when R^2 = Me,Et. The *trans* configuration resulted as a consequence of the steric interactions

between the large *t*-Bu group and R^2 being reduced to a minimum. The hydrolysis of **172** *via* heating with concentrated hydrochloric acid gave the 2,3-methanoamino acids (**173**) in good yield (68%).

Scheme 52



174 **176** **177**

175

176 **178**

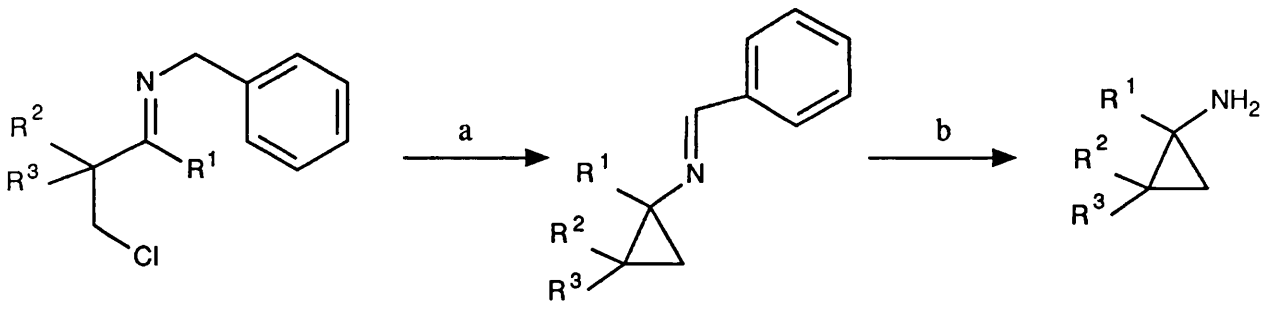
Reagents : (a) KCN , MeOH , Δ ; (b) 6N HCl

(e)Via cyanide additon to α-chloroketimines or base-induced cyclisation of β-chloroimines.- As a result of early research [257] into the preparation of α-cyano-aziridines, Dekimpe *et al* developed a synthesis [258,259] of racemic 1-(alkylamino)cyclopropane carbonitriles (**176**) (Scheme 52). This involved the treatment of tertiary α-chloroketimines (**174**) with potassium cyanide *via* the trapping

out of the intermediate cyclopropylideneamine (**175**) with cyanide ion, generating **176** in 90% yield, together with a small amount of aziridine by-product (**177**). The cyclopropanes (**176**) were hydrolysed under acidic conditions to generate the 2,2-dialkyl-2,3-methanoamino acid (**178**).

An alternative preparation of **178** was later reported by Dekimpe *et al* [260]. This involved a base-induced intramolecular cyclisation of a β-chloro-N-benzylimine (**179**), yielding the N-(benzylidene)cyclopropylamine (**180**) which can be readily hydrolysed into the corresponding cyclopropylamine (**181**). Thus, providing the R^1 substituent is transformable into a carboxyl function, then **181** can be converted into the 2,3-methanoamino acid (**178**) (Scheme 53).

Scheme 53



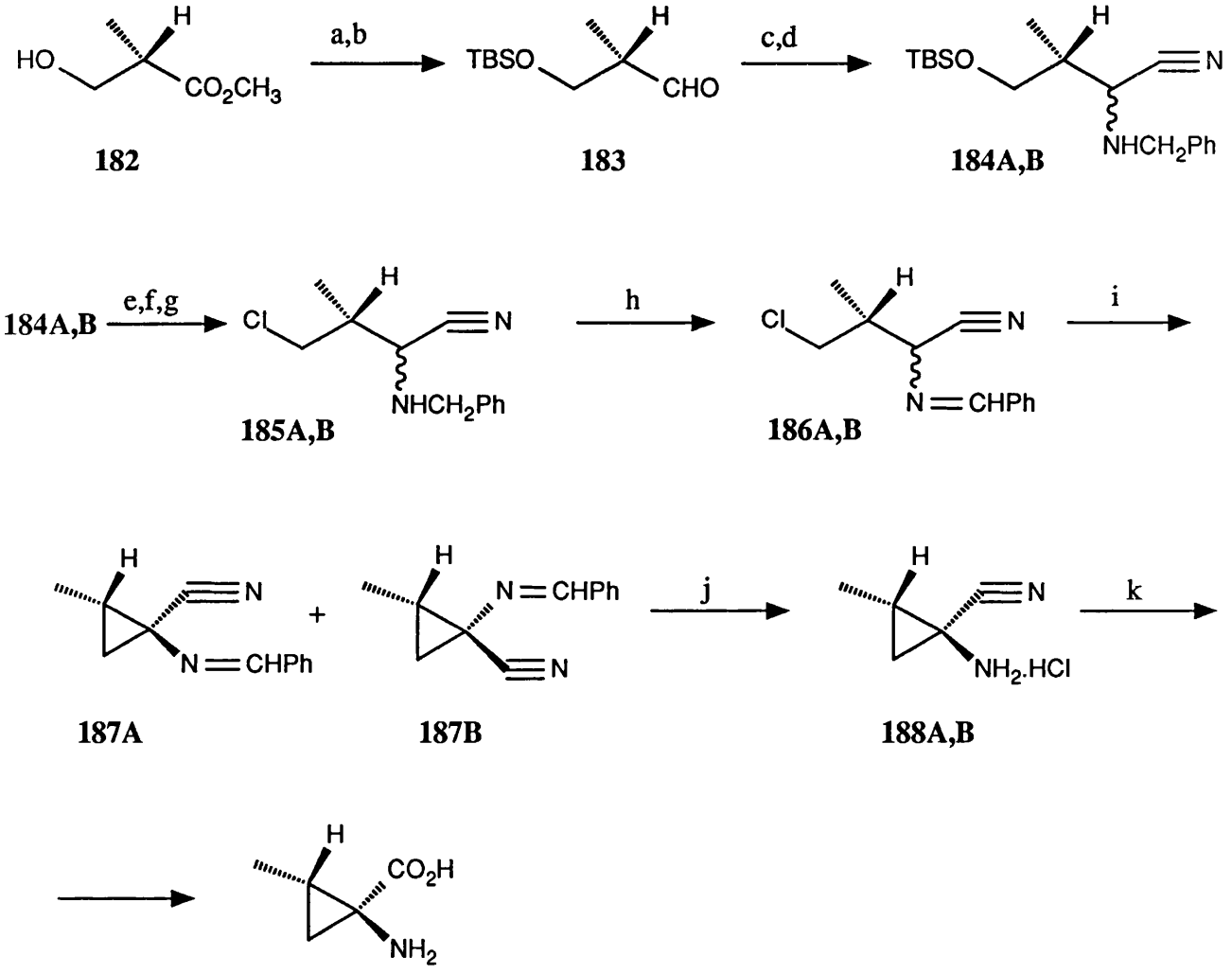
179 **180** **181**

Reagents : (a) *t*-BuOK , THF , Δ ; (b) H_3O^+

A similar cyclisation has more recently been reported by Salaun *et al* [220] in the preparation of norcoronamic acid. The δ-chloronitrile (**185**), resulting from the addition of TMSCN/benzylamine to the aldehyde (**183**), underwent Swern N-oxidation to give the racemic chloroimine (**186A,B**) in 70% yield. The treatment of **186A,B** with base resulted in cyclisation to give the cyclopropanecarbonitrile (**187A,B**) as a mixture of

diastereomers (1S, 2S):(1R, 2S) (84:16) respectively. The mix of diastereomers (**187A,B**) was hydrolysed to afford norcoronamic acid (**189A,B**) and resolved using porcine kidney acylase to give the (1S,2S) diastereomer of **189** in high enantiomeric excess (>95% ee) (Scheme 54).

Scheme 54



Reagents : (a) TBSCl , imidazole , DMF ; (b) DIBAH , DCM , -78C ; (c) TMSCN , ZnI_2 ; (d) $PhCH_2NH_2$, MeOH ; (e) *n*-Bu_4NF , THF ; (f) TsCl , Et_3N , DMAP ; (g) LiCl , N-methyl pyrrolidine; (h) DMSO , $(COCl)_2$, Et_3N , DCM ; (i) K_2CO_3 , DMF ; (j) 1N HCl ; (k) NaOH , Δ .

Peptides Containing 2,3-Methanoamino Acids

2,3-Methanoamino acids have been incorporated into several peptides and their properties studied. The first reported synthesis was that of Tyr-ACC-Gly-Phe-Leu, an analogue of Leu^5-enkephalin [261], in which ACC replaced a glycine residue. In that investigation, the ease of coupling at the ACC amino and carboxyl functions was compared with that of 2-methylalanine (Aib). It was found that the ACC amino and carboxyl groups are more sterically hindered than those of normal α-amino acids, but less than those of Aib. ACC underwent blocking, deblocking and coupling reactions uneventfully.

Another bioactive peptide containing ACC, ACC^7-oxytocin, was synthesised [262] with the finding that sodium/ammonia (liq) deblocking of the cysteine sulphur atoms led to reductive opening of the cyclopropane ring. Whereas, if anhydrous hydrogen fluoride was used, the ring remained intact. This peptide analogue was found to have lower bioactivity than oxytocin.

In 1987, it was reported [263] that the two dipeptide derivatives, N-benzoyl-ACC-Phe-OH (**190**) and N-benzoyl-ACC-Pro-OH (**191**) showed time dependent inhibition of carboxypeptidase A. Thus incubation of the phenylalanine peptide (**190**) caused complete inactivation of the enzyme ($t_{1/2}$ = 4.5 min.) with an apparent $K_i = 8.4 \times 10^{-4}$M. The inhibition occurred about 2.3 times faster than hydrolysis of the peptide. The proline peptide (**191**) was more potent, having a $t_{1/2}$ for total inactivation of 3 min. and an apparent K_i of 5.5×10^{-4}M with no hydrolysis occurring, as expected. Using molecular graphics, it was postulated that the Zn^{2+} ion, known to be present at the active site of carboxypeptidase A, coordinated with the peptide carbonyl group; thus assisting the attack of the Glu^{270} carboxylate anion, an enzyme-situated nucleophile, on the cyclopropane ring leading to covalent bonding with the peptide and irreversible inactivation.

The structural preferences of simple ACC containing peptides have been indicated through X-ray diffraction studies and conformational energy calculations. Semi-empirical potential energy calculations performed on a simple ACC dipeptide and a 2-substituted ACC dipeptide indicated that different types of helices are energetically favoured [264]. Potential energy maps showed that β-substituents may restrict either the ϕ or ψ values selectively. Using both empirical and *ab initio* methods, the conformational effects of introducing ACC into a peptide chain have been studied [265-267]. The concensus of this work indicated that the ACC residue is quite different from the aminoisobutyric acid (Aib) residue, since it favours folding of the peptide chain by formation of a C_7-helix or γ-turn, with formation of a β-turn being a close second energetically. More recently, tri- and tetra-ACC peptides have been shown to fold into distorted type I β bends and 3_{10} helices, in contrast to the acyclic Aib, α,α-diethyl-and α,α-dipropylglycine residues which favour the formation of regular type III β-bends and 3_{10}-helical structures [268-270].

The two amino acids (-)(2S, 3R)- and (+)-(2R,3S)-(E)-2,3-methanophenylalanine have been incorporated into anto an enkephalin analogue, forming [D-Ala^2, Leu^5, (-)-and (+)-∇^EPhe^4]enkephalins. These peptides were reported to show reduced activity in the mouse vas deferens (MVD) and guinea pig ileum (GPI) muscle assays [271]; the peptide showing a strong preference for the δ-receptor of the GPI. It was later found that the peptide containing the (2R, 2S)-isomer showed a very high preference for the δ-receptor in rat brain, while the other peptide was inactive [272]. Thus indicating a possible differentiation between the peripheral and central nervous system receptors by these peptides.

During the synthesis of a series of aspartyl dipeptides containing a 1-amino-1-cyclopropanecarboxylic acid at the carboxyl terminus, it was found that

Asp-ACC-OMe had a distinct sweet smell [273]. Further work [274], on a series of esters Asp-ACC-OMe showed that the *n*-propyl ester had 250-300 times the sweetness potency of sucrose. More recently, all four diastereomers of Asp-∇Phe-OMe were prepared [275] with the expectation that at least one of the four compounds would be sweet and allow the close mapping of the "sweet receptor". Surprisingly all of these compounds were tasteless. Molecular modelling, CD and NMR studies of these dipeptides led to the conclusion that the rigid positioning of the phenyl moiety by the cyclopropane ring prevented its necessary orthogonality [276] to the flat zwitterionic ring formed by the aspartic acid residue, thus prohibiting their binding to the taste receptor.

The incorporation of (Z)-2,3-methanophenylalanine into the natural chlorosis-causing peptide tentoxin, cyclo[N(Me)Ala-Leu-(Z)-N(Me)-Δ^{Z}Phe-Gly], by replacing the dehydrophenylalanine residue, led to a peptide with no bioactivity in the lettuce seedling assay [277]. Tentoxin, a secondary metabolite of *Alternaria alternata*, is of agricultural interest because it selectively affects susceptible weeds while ignoring major crop plants.

1.5 Peptide Isoteres

Enzyme Action and Inhibition

The function of enzymes is to act as catalysts in biological reactions, although often a further non-protein component called a cofactor is required before an enzyme has catalytic activity. Enzymes can catalyse reactions by virtue of the amino acid residues present in their chains. Every enzyme has a unique protein structure defined by a characteristic amino acid sequence. This makes them substrate specific.

Catalysis is the result of raising the ground state energy for the reaction. It is possible to make some generalizations concerning the methods by which enzymes catalyse reactions:

(a) Deforming a configuration of atoms away from their equilibrium position raises the energy of the system. If an enzyme can stretch a bond, the energy will become closer to the transition state energy and bond cleavage will be aided. In order to strain a system there must be some interaction of that system with the enzyme, equal in energy to the induced strain energy.

(b) Enzymes which can concentrate and orientate reactants will catalyse reactions. A simple bringing together of reactants can theoretically cause an acceleration, largely due to the change in concentration caused.

(c) Nucleophilic attack on an electrophile can be speeded up by making the electrophile more electrophilic and vice-versa. This is electronic activation.

Enzymes bind substrates at a region of the enzyme termed the active site. Binding sites link to specific groups in the substrate, fixing its relative orientation, so that reacting

groups are in the vicinity of catalytic sites on the enzyme. Inhibitors are substances that tend to decrease the rate of an enzyme catalysed reaction. Some inhibitors act on the substrate or cofactor, but this discussion is concerned only with those which combine directly with the enzyme.

There are several forms of enzyme inhibition: competitive, uncompetitive, non-competitive, as well as mixed, partial, and allosteric.

Competitive inhibitors often closely resemble the substrates whose reactions they inhibit, and therefore compete for the same binding site on the enzyme. The enzyme bound inhibitor then either lacks the appropriate reactive group or is held in an unsuitable position with respect to the catalytic site. A dead-end complex is formed and the inhibitor must dissociate from the enzyme and be replaced by a molecular substrate, before a reaction can take place. Uncompetitive inhibitors bind only to the enzyme-substrate complex and not to the free enzyme. Substrate binding could cause a conformational change to take place and reveal an inhibitor binding site, or the inhibitor could bind directly to the enzyme-bound substrate, both resulting in the formation of a dead-end complex. Non-competitive inhibitors combine with an enzyme molecule to produce a dead-end complex, whether a substrate molecule is bound or not. The inhibitor must, by definition, bind to a different site to the substrate, destroying the catalytic activity of the enzyme.

Irreversible inhibitors bind to the active site of the enzyme, usually by formation of a covalent bond. The inhibitor may act by preventing the substrate binding or it may destroy some component of the catalytic site. An inhibitor which shows great affinity for the enzyme is regarded as irreversible.

Any data discussed in the text will be associated with enzyme inhibition *in vivo* by

various compounds synthesized and will be represented in terms of binding affinities, K_i, or IC_{50} values, where IC_{50} is the concentration required to produce half maximal enzyme inhibition.

Peptide Mimetics as Enzyme Inhibitors

In recent years extensive work has been performed on peptide mimetics [278,279]. Analogues of natural peptide substrates have been prepared in which conformational constraints and non-peptide linkages have been introduced, as well as by changing the natural amino acid sequence and making amino acid side chain substitutions. Structural modifications are introduced into the parent compound to determine the resulting biological consequences. This discussion covers peptide backbone modifications only. Peptide backbone studies have attempted to identify the importance of rigidity, alignment and stereochemistry for biological function and also how modification of the backbone affects resistance towards enzymatic degradation.

The introduction of peptide backbone modifications can be used in the design of potent peptide analogues or, conversely, peptide antagonists. An antagonist is a compound that binds to a receptor but elicits no response. The peptide backbone is made up of three repeat elements; the amide N, α-C and amide carbonyl. These have all been subject to replacement in the course of new analogue design, either individually or in various combinations. The amide group represents a single replaceable unit. The symbol ψ[ ] is used to show that the natural peptide unit has been replaced by the unit inside the bracket. Refer to Table 8.

As with side-chain modifications, structural similarity to the natural peptide is not necessarily essential for biological potency. Subtle changes in bond length and angles could improve receptor interactions. In addition to possessing enhanced resistance to biodegradation, many amide bond surrogates possess lipophilic, electronic and

hydrogen-bonding properties which can affect transport, receptor binding or membrane interactions. In the search for potent enzyme inhibitors, requirements are for receptor selective antagonists with a prolonged activity profile. Potency and selectivity vary with the peptide backbone modification and its position within the peptide chain. Every enzyme receptor is specific, demanding agonists and antagonists with varying structural and electronic properties for strong coordination and resistance to biodegradation.

In order to confer stability to a hormone analogue against rapid degradation, scissile bonds are identified in the parent molecule and then replaced by a surrogate offering greater resistance. A scissile bond is the bond in a substrate subject to enzymatic cleavage. The introduction of D-amino acids, N-methyl amino acids, α-substituted amino acids into peptides gives mimetics with partial or full enzymatic resistance. This effect must be due to altered conformational or electronic properties that decrease the fit of the modified analogue, since the cleavage link is still present. A common mechanistic feature of many proteolytic enzymes appears to be polarization of the amide carbonyl, followed by nucleophilic attack on the weakened scissile bond. A proteolytic enzyme catalyses the hydrolysis of peptide bonds. In principle, any backbone modification which removes the polarizable group, alters the geometrical requirements and/or otherwise increases the strength of the vulnerable link, will tend to reduce or eliminate the possibility of proteolytic degradation.

Linear peptides usually assume multiple low-energy conformers. Conformation induction and selection have been recognised as being important in hormone receptor binding. It has been proposed that conformational changes take place during or after the binding event. This is supported by the finding that relatively rigid analogues often prove to be good antagonists. Conformationally constrained analogues have been designed by the use of peptide backbone modifications, from which it is evident that

conformational effects are important in the design of potent agonists.

Table 8

Modifications of the Peptide Backbone Involving the Amide Nitrogen	
N-methyl	ψ[CON(CH_3)]
N-hydroxy	ψ[CON(OH)]
keto methylene	ψ[$COCH_2$]
ester	ψ[COO]
thioester	ψ[COS]

Modifications of the Peptide Backbone Involving the α-Carbon	
normal amide	-CH(R)CONHCH(R^1)-
α-methyl	-CH(R)CONHCH(R^1)(CH_3)-
α,α-disubstituted	-CH(R)CONHC(R^1)(R^2)
α-aza	-CH(R)CONHN(R^1)-
dehydro amino acids	-CH(R)CONHC(=CR_2)-
α-bora	-CH(R)CONHCB(R^1)-
cyclopropylogs	-CH(R)CONH 

Also invertion of the natural amino acid residue stereochemistry at α-C

Modifications of the Peptide Backbone Involving the Amide Carbonyl	
reduced carbonyl	ψ[CH(R)NH]
thioamide	ψ[CSNH]
sulphonamide	ψ[SO_2NH]

Modifications of the Amide Bond

carba	$\psi[CH_2CH_2]$
ethylenic	$\psi[CH=CH]$
	$\psi[CH_2X]$ X=S,O
retroinverso	$\psi[NHCX]$
retro-aminomethylene	$\psi[NHCH_2]$
	$\psi[C(=CH_2)CH_2]$
acetylene	$\psi[C\equiv C]$

Modifications of the Peptide Backbone Involving Extensions or Multiple Replacements

aza-extensions	$\psi[NHCONH]$
aminoxy	$\psi[CONHO]$
tetrazole	$\psi[CN_4]$
methylene hydroxyamino	$\psi[CH_2N(OH)]$
nitrono	$\psi[CH=N^+\text{-}O^-]$
lactams	$\psi[CHCON]$ with a bridge from CH to N through X

Also β-aminoacids in place of natural residues and *p*-amino methyl benzoic acid extensions.

1.6 Molecular Dynamics

Molecular Dynamics procedure is a deterministic simulation process wherein the positions and velocities of atoms in a molecule are integrated forward in time, using Newton's laws of motion. The initial velocities are randomly ascribed to atoms *via* a Maxwellian distribution consistent with the temperature at which the simulation is being performed. The movement of the atoms is then governed by the kinetic energy input into the system and the restoring forces that act on the molecule when its position from a minimum energy conformation is disturbed. The latter term is a forcefield term from which the potential energy of the system can be determined. This term consists of strain energies like bond length, bond angle deformations, torsional components *etc.* and interaction terms like van der Waals, non-bonded interactions and electrostatic terms. In this thesis, the Valence Force Field (VFF) is used to compute potential energy and the expression is given below (Eq.1). The potential energy of the compound studied is represented by the full valence force field (VFF).

$$
\begin{aligned}
V \quad &= \Sigma\{D_b[1-e^{-\alpha(b-bo)}]^2-D_b\} \qquad \textbf{(Eq.1)} \\
&+ 1/2\ \Sigma H_\theta(\theta-\theta_o)^2 \\
&+ 1/2\ \Sigma H_\phi(1 + s\cos n\phi) \\
&+ 1/2\ \Sigma H_\chi\chi^2 \\
&+ \Sigma\Sigma F_{bb'}(b-b_o)(b'-b_o') \\
&+ \Sigma\Sigma F_{\theta\theta'}(\theta-\theta_o)(\theta'-\theta_o') \\
&+ \Sigma\Sigma F_{b\theta}(b-b_o)(\theta-\theta_o) \\
&+ \Sigma F_{\phi\theta\theta'}\cos\phi(\theta-\theta_o)(\theta'-\theta_o') \\
&+ \Sigma\Sigma F_{\chi\chi'}\chi\chi' \\
&+ \Sigma\epsilon[r^*/r)^{12}-(r^*/r)^6] + \Sigma q_i q_j/r
\end{aligned}
$$

The first four terms define the energy required to distort the internal from their ideal values. (b, Θ, ϕ and χ are the bonds, valence angles, torsion angles and out of plane angles, respectively; b_o and Θ_o are the ideal bond lengths and valence angles and the H terms are the force constants for distoring the internals). The last three terms represent the interactions between non-bonded atoms (r* is the most favourable interatomic distance and ϵ the energy of interaction at that distance, q_{ij} are the partial atomic charges). Cross terms representing coupling between two or more internals are also included. These are required for reproducing experimental vibrational data and will affect the shape of the energy surface in non-equilibrium regions. The parameters in the equation 1 [280] were derived by fitting experimental data for small model compounds including functional groups that occur in amino acids. The non-bond parameters were obtained by fitting crystal structure sublimation energies and dipole moments [281,282]. These parameters were validated against high grade quantum mechanical calculations [283] and tested for conformation dependent properties in solution [284]. The valence parameters were obtained by fitting vibrational spectra, molecular structures and rotational barriers [285].

The flexible geometry ϕ,ψ energy maps were calculated using the full VFF expression and adding two forcing terms:

$$E_\phi = k_\phi(\phi-\phi_o)^2 \qquad \textbf{(Eq.2)}$$

$$E_\psi = k_\psi(\psi-\psi_o)^2$$

A force constant of 1000kcal/mol was used in order to force the angles ϕ and ψ to adopt conformation (ϕ_o,ψ_o), while minimising the total energy to relax all other degrees of freedom. The values of ϕ_o and ψ_o were incremented by steps of 10-20°, to produce the complete ϕ,ψ map. The minimisation for each map point was carried out in two stages: first a steepest descent minimisation was carried out to relieve initial clashes and then a

quasi Newton method [286] was used to achieve convergence. (Achieved when the largest derivative of the energy with respect to any Cartesian coordinates was smaller than 0.0001 kcal/Å). Grid points that correspond to local minima were subject to further minimisation, without the torsion constraints, to reveal the exact location and energy of the minima.

Isolated β sheets and turns do not always correspond to a local minimum. Thus in addition to the full VFF expression two types of forcing terms were added, one of which ensures the backbone conformation is not changed drastically by applying torsion forcing terms (Eq. 2). In addition the typical hydrogen bonding pattern is preserved by adding a small penalty function:

$$E_{hb}=k_{hb}(r_{ij}-r_{ij}^{o})^2 \qquad \textbf{(Eq.3)}$$

Where r_{ij} is the distance between a pair of hydrogen bonded atoms. A force constant of 5kcal/mol was used in order to force the angles ϕ and ψ to adopt conformation (ϕ_o,ψ_o), and a force constant of 2kcal/mol was used to preserve the hydrogen bonds, while minimising the total energy to relax all other degrees of freedom.

The software packages DISCOVER and INSIGHT (BIOSYM Inc. USA) were used to perform molecular dynamics simulations and energy minimisation studies. Plots of molecular conformations were done by ORTEP [287].

Accessible conformations of Δ^{Z}Phe and $\nabla^{Z/E}$ peptides were obtained by performing constrained molecular dynamic simulations using types, I, I', and II' β turns as four starting points in the search. The simulation periods were over 50ps each for the Δ^{Z}Phe peptide and 25 ps each for the $\nabla^{Z/E}$ peptide. Thus a total of 300 ps of simulations were

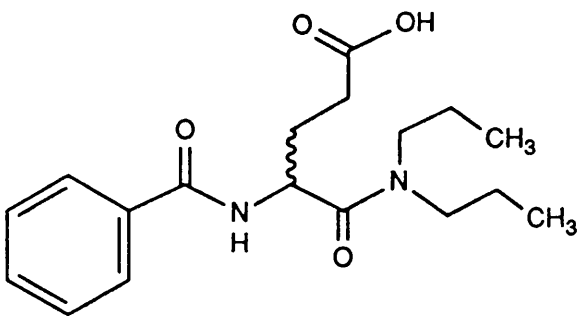
performed. The resulting conformations were minimised without any constraints. For the $\nabla^{Z/E}$ peptide, proper minimisation was found possible only when a harmonic potential was used to describe the bond lengths and the cross terms were turned off in the energy function.

1.7 Cholecystokinin and Cholecystokinin Antagonists

Cholecystokinin (CCK) is found in several peripheral sites where it acts as hormonal regulator of gut function, digestion and feeding [288,289]. It is also found in the nervous system where it functions as a neurotransmitter and a neuromodulator [290,291]. Several biologically active forms of cholecystokinin exist, the prevalent forms in the periphery being CCK-58, CCK-33, CCK-8 (H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2) and CCK-4 (H-Trp-Met-Asp-Phe-NH_2). CCK is found in the central nervous system (CNS) predominantly as CCK-8. The cerebral cortex, the limbic system, midbrain and spinal cord neurons contain high levels of CCK peptides relative to other neuropeptides. CCK-8 coexists with dopamine in mesolimbic and mesocortical pathways and with Met-enkephalin in hypothalmic, hippocampal and periaqueductal regions, CCK's role in satiety and feeding behaviours, its coexistence with other neurotransmitters and neuropeptides, and its relative abundance in the CNS compared to other neuropeptides, has made it the focus of considerable research effort over the past decade.

The CCK receptor family has been subdivided into two types. Namely the CCK-A (alimentary) receptors which predominate in the periphery and are characterised by the CCK receptors in the gall bladder, pancreas and ileum; and CCK-B (brain) receptors that predominate in the CNS and are typically characterised by receptors in the cerebral cortex [292]. CCK-A receptors have been identifed in the CNS in several discrete brain regions such as the *nucleus tractus solitarius* and the *dorsomedial caudate* [293]. The role of CCK-A receptors in the CNS is presently unknown although preliminary evidence indicates that a possible role is in the modulation of dopaminergic function [294,295]. Similary, the functional significance of brain CCK-B receptors is unclear; although recent data suggests a role in the modulation of pain [296], anxiety [297],

cognition [298] and perhaps the regulation of certain feeding behaviours [299]. The CCK-B receptors have also been reported in peripheral sites [300] but controversy remains over whether these receptors are indeed of CCK-B type or gastrin receptors. The structural similarity of gastrin and CCK peptides, and the inability of ligands, either peptidic or non-peptidic, to differentiate gastrin from CCK-B receptors warrants the inclusion of gastrin and its receptors into this discussion. Before the existence of receptor subtype specific probes, these two classes were differentiated by their relative affinities for CCK-4, CCK-8 and its desulphated form (CCK-8(DS)). The CCK-B/gastrin receptors recognise all of these with comparable affinity, whereas only CCK-8 is bound with high affinity by the CCK-A receptor.



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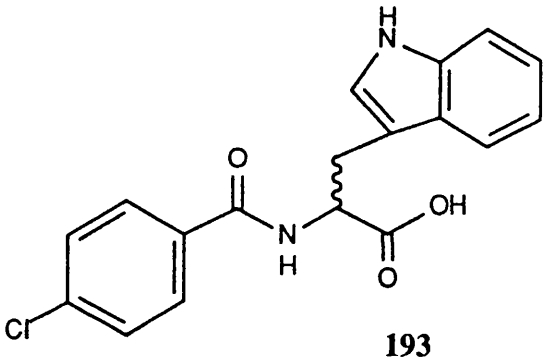

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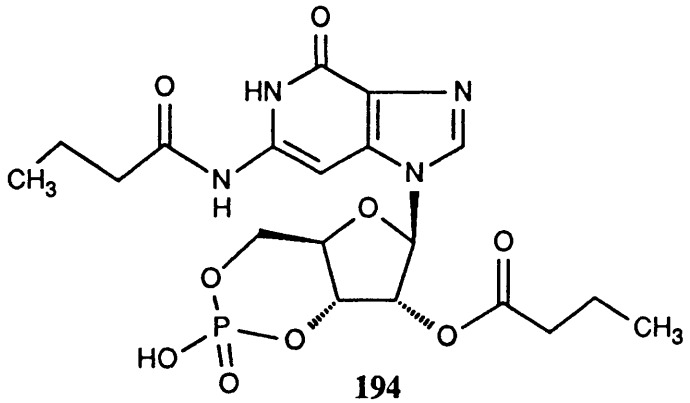
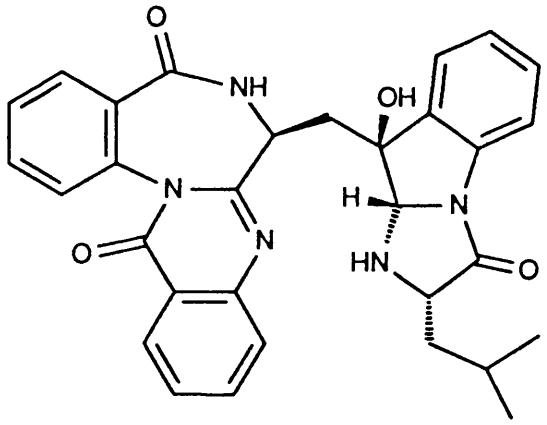


194

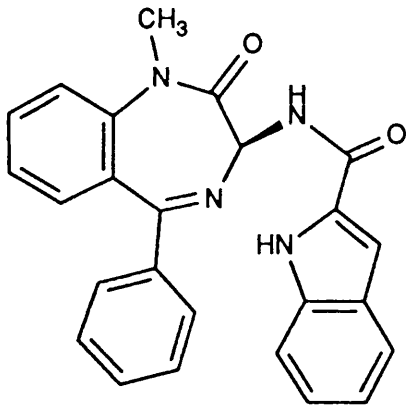
Prior to the recent advances in CCK research, a number of weaker and non-selective CCK antagonists such as proglumide (**192**), benzotript (**193**), and dibutyryl-cyclic GMP

(**194**) were utilised to elucidate CCK pharmacology.

These antagonists have been well studied and previously reviewed [301,302]. The discovery of asperlicin (**195**), a fermentation product from *Aspergillus alliaceus* [303], initiated the synthesis of similar benzodiazepine derivatives. This resulted in the discovery of the potent CCK-A selective agent devazepide (**196**), also known as MK-329 [304], which has an affinity for CCK-A receptor comparable to CCK itself ($IC_{50} = 10^{-10}M$) in a variety of animal models [305]. Further efforts provided the potent CCK-B/gastrin selective compound (R)-L-365,260 (**197**) [306].



195



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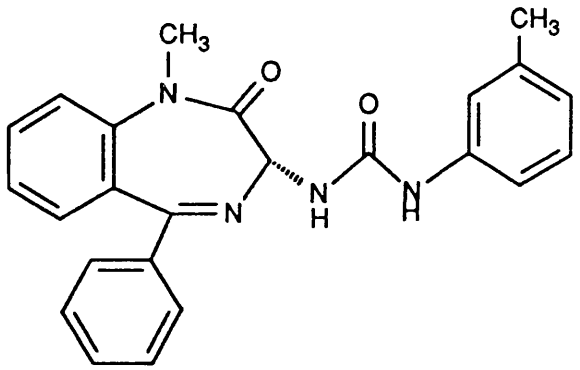


197

The enantiomer of **197** displays selectivity for the CCK-A receptor just as devazepide does. In fact the stereoinversion of the chiral centre in these benzodiazepines was

found to be crucial to the strategy for developing a CCK-B/gastrin selective agent. Both devazepide and (R)-L-365,260 represented major advances in the CCK antagonist field in that they were both potent and selective with good oral bioavailibility.

Peptide Antagonists

A considerable amount of research within the CCK field has centred on analogues of the parent peptides CCK-8 and CCK-4. It should be noted, however, that within this subclass the difference between agonism and antagonism is sometimes not well defined owing to some entities possessing mixed functional responses, or showing species dependent pharmacology.

The general strategies involved in the preparation of peptide antagonists have included:-

(a) Amino acid replacement [307]

(b) Substitution of the amide bond (CONH) by isosteric equivalents, e.g. ketomethylene ($COCH_2$) [308], aminomethylene (CH_2NH) [309], carba (CH_2CH_2) [310], ethylene (CH=CH) [311,312] and retro-inverso amides (NHCO) [313,314]

(c) The cyclisation of linear peptides [315]

(d) The incorporation of conformationally restricted amino acids [316].

These efforts have resulted in some ligands with very high affinities for either CCK-A or CCK-B/gastrin receptors. One of the earliest findings [317] was that deletion of the C-terminal phenylalanine amide would lead to a series of peptide antagonists such as compound (**198**).

Cbz-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-NH_2

198

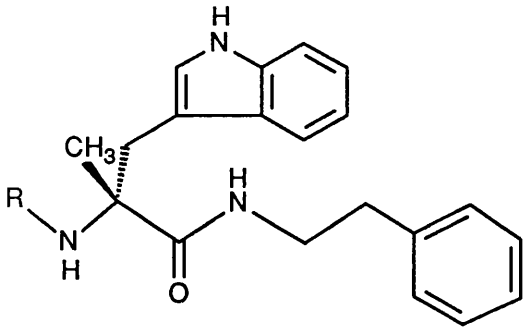
Although it was shown that the antagonism of **198** was species dependent [318], the removal and/or modification of the C-terminal portion of CCK peptides has remained a viable approach to pharmacologically interesting agents [319]. The des-carboxamide CCK analogue JMV-180 (**199**), for example, was found to act as an agonist at high affinity rat pancretic CCK receptors where it stimulates amylase release.

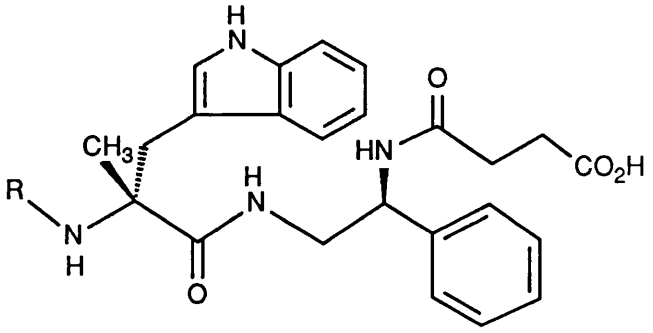
Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-O(CH_2)$_2$Ph

199

CCK antibodies, being peptidic in nature, represent a further approach to achieve CCK antagonism. Their use is of current interest in the promotion of weight gain in livestock. A recent study has appeared describing the immunisation of livestock with CCK for this purpose [320].

Peptoid Antagonists





200 , R= 1 -Adamantyloxycarbonyl (1-Adoc)

201 , R= 2-Adoc
202 , R= 1(S) - Endobornyloxycarbonyl

A number of research groups have used CCK-4 as the basis for further exploration of high affinity CCK-B ligands. Utilising a deletion approach Horwell and co-workers [321] initially evolved a series of "dipeptoids" represented by the tryptophan analogue (**200**), with moderate affinity (IC_{50}≈1-10μm) for the CCK-B receptor.

This approach addressed two of the major problems associated with peptide- based therapeutic agents. First, by decreasing the overall molecular size it allowed the possibility of better adsorption, and second, by specifically using α-methyl-D-tryptophan it greatly enhanced metabolic stability. Further research aimed at re-incorporating functional groups from CCK-4 led to the discovery of the novel dipeptoids PD-134308 (Cl-988) (**201**) possessing a greatly enhanced potency at CCK-B receptor (IC_{50}=1.7nM) [322] and its closely related analogue PD-135138 (**202**) [323].

CHAPTER 2

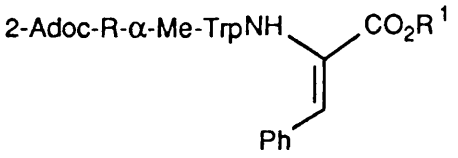
RESULTS AND DISCUSSION

DISCUSSION

2.1 Aims and Objectives

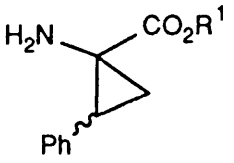
The objective of this project was as follows :-

a, the preparation of dehydrophenylalanine mimetics (**203**) for CCK-4 [H-Trp-Met-Asp-Phe-NH_2]



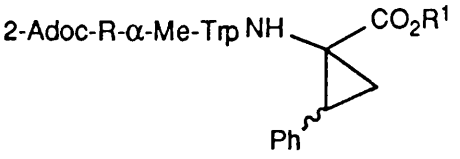
203

b, the diastereoselective synthesis of 2,3-methanophenylalanine esters (**204**)



204

and c, the inclusion of **204** into the corresponding CCK dipeptide analogues (**205**)

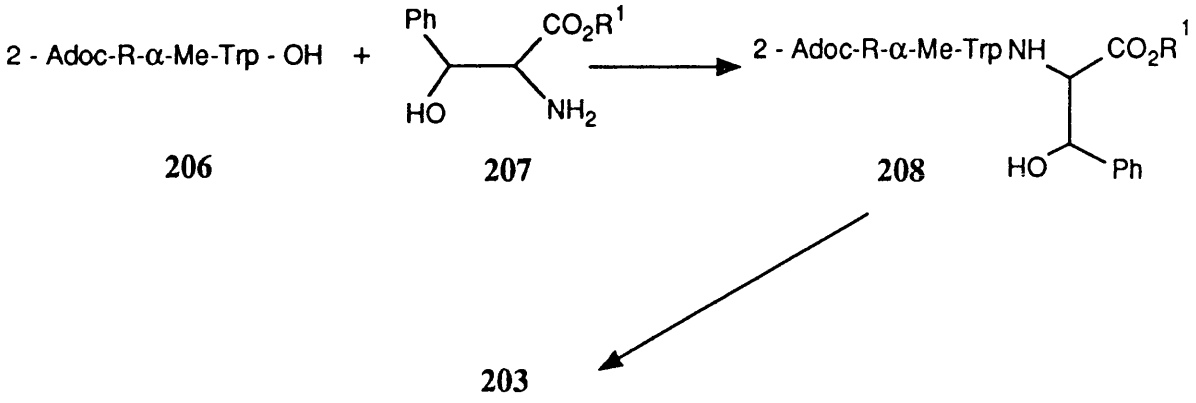


205

The strategy employed in the preparation of the dehydropeptides (**203**) would involve the coupling of an N-blocked tryptophan (**206**) to the (RS)-*threo*-3-phenylserine ester

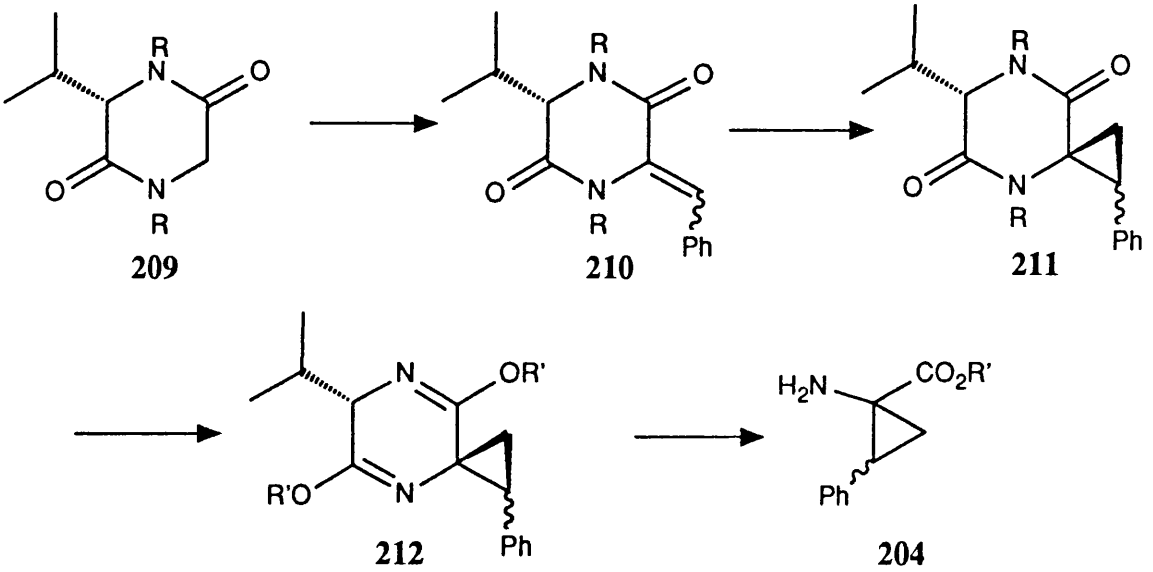
(**207**) followed by dehydration of the alcohol(**208**) (Scheme 55).

Scheme 55



For the synthesis of 2,3-methanophenylalanine ester (**204**) a chiral diketopiperazine template (**209**) would be employed, which once modified to the benzylidene (**210**) *via* condensation with benzaldehyde would induce selective cyclopropanation. Then on suitable modification of the diastereomerically pure cyclopropanes (**211**) would allow the isolation of the desired amino esters (**204**) (Scheme 56).

Scheme 56



The esters (**204**) would then be similarly coupled to the N-blocked tryptophan (**206**) to

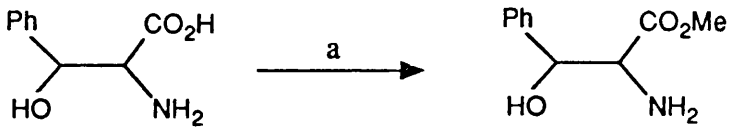
generate the cyclopropyl dipeptide analogues (**205**).

2.2 Preparation of dehydrophenylalanine dipeptide derivatives

Preparation of (RS)-threo-3-phenylserine methyl ester (**214**)

The esterification of the acid, (RS)-*threo*-3-phenylserine hydrate (**213**), to the methyl ester (**214**) employed conventional esterification techniques [324]. Thus, the treatment of **213** with methanolic thionyl chloride gave, after basic work-up, the ester (**214**) uneventfully in 80% yield (Scheme 57).

Scheme 57



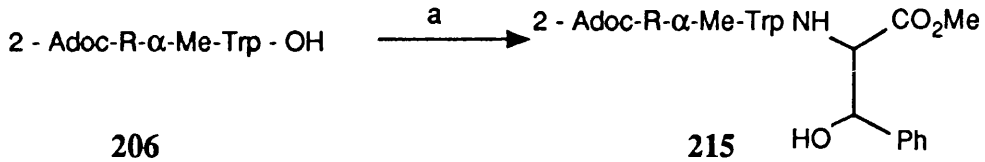
213 **214**

Reagents : (a) i) $SOCl_2$, MeOH , 0°C, ii) 1M NaOH , pH 7 , 0°C .

Preparation of N^{α}*-(2-adamantyloxycarbonyl)-α-methyl-R-tryptophanyl-RS-threo-3-phenylserine methyl ester* (215)

The coupling of the N-protected tryptophan (**206**) with the C-protected RS-*threo*-3-phenylserine (**214**) was performed by prior activation of the acid using HOBt/DCC. These coupling agents were chosen since they have been reported to give good ester yields, but more importantly, with the minimal occurence of racemisation [325,326]. The desired dipeptide ester (**215**) was successfully prepared in 83% yield as a mixture of RS-*threo*-enantiomers (Scheme 58).

Scheme 58

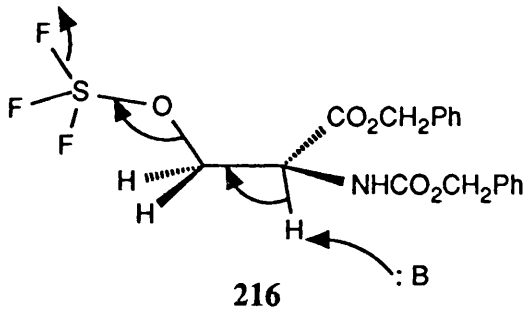


206 **215**

Reagents : (a) i) DCC , HOBT , EtOAc ; ii) **206** , EtOAc .

*Dehydration of the alcohol (***215***) to yield the dehydroamino acid* N^{α}*-(2-adamantyloxycarbonyl-α-methyl-R-tryptophanyl-dehydrophenylalanine methyl ester* (**219**)

As was discussed in Section 1.3, various dehydrating reagents have been employed successfully for the preparation of dehydroamino acids from β-hydroxy-α-amino acids, *via* β-elimination reactions.



216

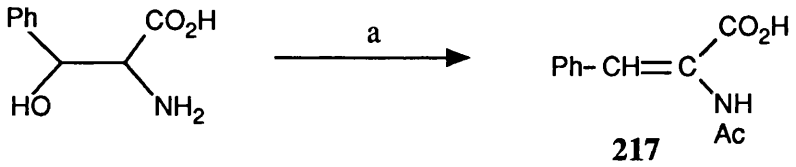
(Diethylamino)sulphur trifluoride (DAST) is generally used in the fluorination of alcohols [327], however, there have been a few reports of dehydration products being isolated [328,329]. In 1983, Somekh and Shanzer reported [330] the steresospecific dehydration of derivatives of serine, threonine (*threo* and *erythro*) and leucine (*threo* and *erythro*) using DAST in the presence of pyridine. They found that *threo*-hydroxy amino acids (**216**) gave (Z)-dehydroamino acids whilst their *erythro* isomers gave the

corresponding (E)-adducts. These results being compatible with an E2 elimination process.

As a result of which we applied these conditions to our system (**215**). Unfortunately no desired dehydroamino ester (**219**) was isolated. The brown colouration of the solution observed, indicated that the reaction conditions were too harsh, so milder conditions were then sought. The treatment of a serine moiety with N,N′-carbonyldiimidazole in the presence of triethylamine was originally employed by Andruszkiewicz and Czerwinski [58] in their preparation of the corresponding dehydroamino esters. These conditions were undertaken on the alcohol (**215**) but this time no colour change was observed and t.l.c. indicated no new product had been formed.

In 1986, Kato *et al* reported [59] the isolation of dehydroserine derivative (**217**) in 90% yield from the β-phenylserine monohydrate *via* treatment with sodium acetate in acetic anhydride (Scheme 59).

Scheme 59

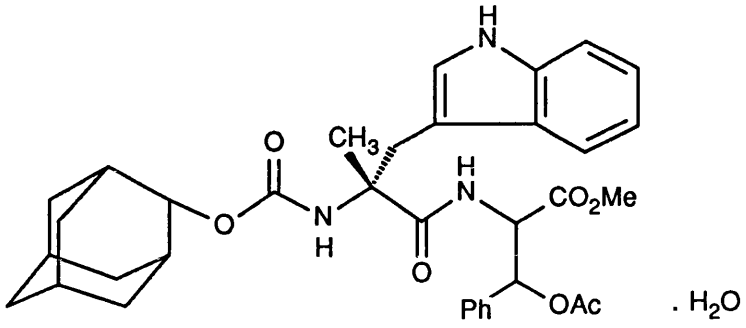


217

Reagents : (a) AcONa , Ac_2O , 15 - 45°C , 5h

These conditions were then applied to our analogous system. This generated a pale yellow solid **218** in quantitative yield. The ^1H NMR spectrum of **218** showed the desired olefin had not been formed but that the intermediate O-acetate had been isolated instead. This was confirmed by microanalysis which revealed **218** to be the

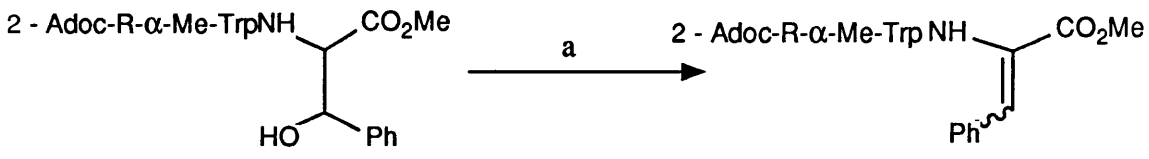
O-acetate monohydrate.



218

Previously, Ogura *et al* reported [331] that N,N′-disuccinimiydl carbonate (DSC) could be used as a coupling reagent in peptide synthesis instead of DCC. Taking advantage of the dehydrating properties of this reagent he later announced [56] that DSC could be used to prepare dehydroalanine and (Z)-dehydroaminobutyric acid from N-blocked-serine and threonine residues, respectively, in good yield. So, to a solution of the alcohol (**215**) in dry acetonitrile was added DSC at room temperature under an atmosphere of nitrogen. After work-up and purification by flash chromatography a new product was isolated as a white foam in 49% yield. This was shown to be the desired dehydroaminodipeptide (**219**) by ^{1}H NMR studies and microanalysis (Scheme 60).

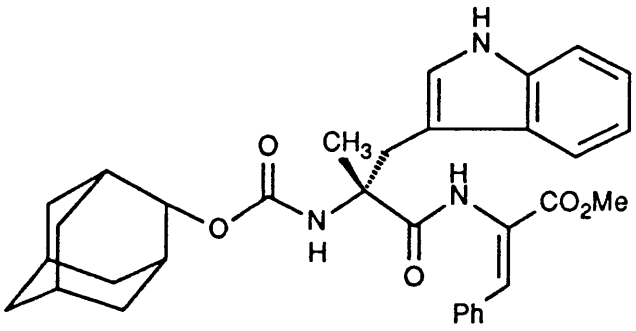
Scheme 60



215 **219**

Reagents : (a) DSC , Et_3N , CH_3CN , room temp

The stereochemistry of the alkene was determined by n.O.e. experiments. Irradiation of the enamine NH (2-NH.2) at δ 8.20 ppm gave no enhancement of the vinyl proton whilst irradiation of the vinyl proton (3-H.2) at δ 7.33 ppm gave the same result. Thus indicating a resultant (Z)-stereochemistry of the phenyl group with respect to the enamine NH as expected.

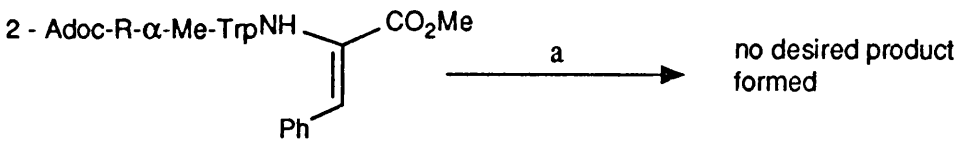


219Z

Attempted hydrolysis of the dehydrodipeptide ester (**219Z**)

Typical hydrolysis reagents were employed in the hydrolysis of the dehydrodipeptide ester (**219Z**). Firstly, dilute lithium hydroxide was attempted. A 0.1M lithium hydroxide solution was added to a solution of the ester (**219Z**) in THF at room temperature. After stirring for a few hours no desired acid was observed. Even refluxing for 12h gave no change (Scheme 61).

Scheme 61

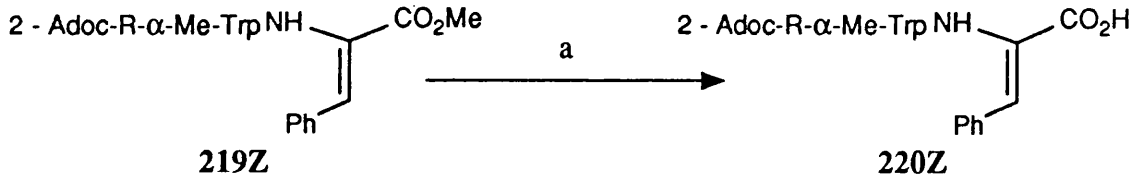


219Z

Reagents : (a) 0.1M LiOH , THF , Δ , 12h

The saponification process was repeated using a different base - 0.1M sodium hydroxide - and to aid solubility a more polar solvent, ethanol, was used. After 2h stirring at room temperature the solution was brought to reflux and after a further 3h complete hydrolysis had been achieved. Purification of the residue by reverse-phase chromatography gave the acid (**220Z**) as a white foam in 30% yield. The ^{1}H NMR spectrum of **220Z** confirmed the structure (Scheme 62)

Scheme 62



219Z **220Z**

Reagents : (a) 0.1M NaOH , EtOH , Δ

The overall forcing conditions were necessary owing to the reduced electrophilicity of the carboxyl carbonyl group as a result of the conjugation to the alkene. The use of a more polar solvent, meanwhile, aided the solubility of the carboxylate salt formed (Scheme 62).

Attempted cyclopropanation of the dehydropeptide ester (**219Z**)

Considerable research has been achieved in the cyclopropanation of derivatised dehydroamino acids and peptides containing them (see Section 1.4). It was felt worthwhile in attempting to produce cyclopropyl dipeptides by this approach.

To a solution of the dehydrodipeptide ester (**219Z**) was added an ethereal solution of diazomethane at room temperature. After stirring for one day a considerable amount of starting material remained and so an excess of diazomethane was added. The reaction

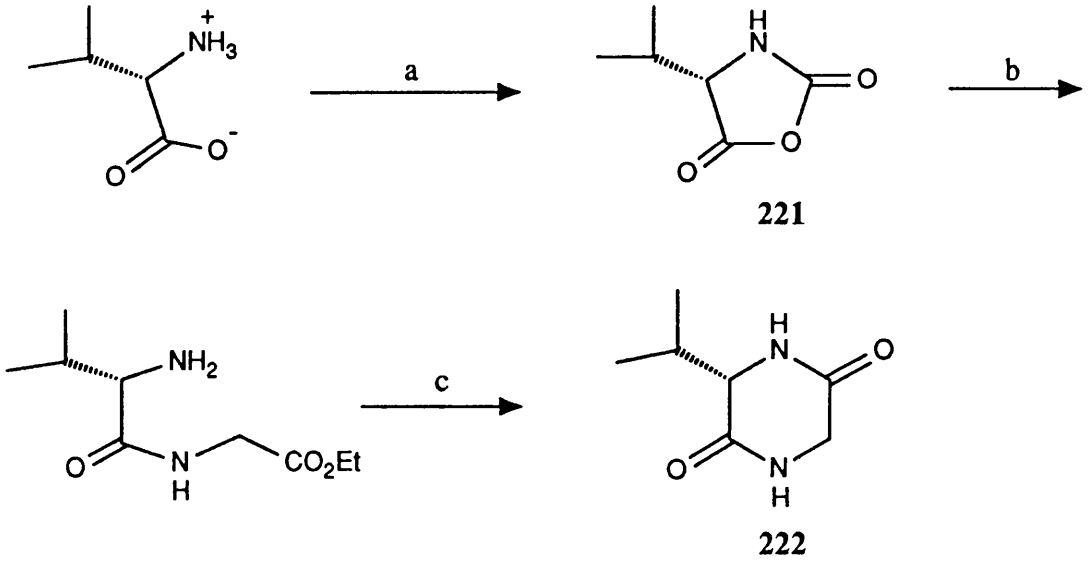
was worked up after a further day. ^1H NMR spectrum of the residue revealed a multicomponent mix, none of which corresponded to the desired cyclopropyl dipeptide.

2.3 Preparation of 2,3-methanophenylalanine derivatives

A diastereoselective synthesis of 2,3-methanophenylalanine derivatives was proposed and, the methodology of which, was based on Schöllkopf's bislactim ether approach [332], whereby we utilised his procedure in the formation of a chiral piperazin-2,5-dione template (**222**) (see Scheme 63).

Preparation of the piperazin-2,5-dione template (**222**)

Scheme 63



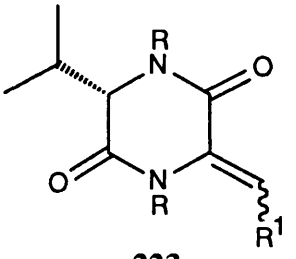
Reagents : (a) $COCl_2$,(20% toluene solution) , THF ,40°C ; (b) $H_2NCH_2CO_2Et.HCl$, Et_3N , THF ,$CHCl_3$, -78°C ; (c) toluene , Δ

The oxazolidine (**221**) was prepared in quantitative yield by the treatment of a suspension of L-valine in THF. The suspension was heated at 40°C until the solution was clear. The careful removal of the solvent, after purging with nitrogen, gave the desired product (**221**) as a white pungent solid (Scheme 63). This unstable compound

was then converted directly into the stable piperazin-2,5-dione *via* the addition of a solution of **221** in THF into a suspension of glycine ethyl ester hydrochloride and triethylamine in chloroform at -78°C with overhead stirring. This gave the desired product (**222**) as a white powder in 60% yield.

Preparation of 6-(S)-1,4-diacetyl-6-isopropylpiperazin-2,5-dione (**226**)

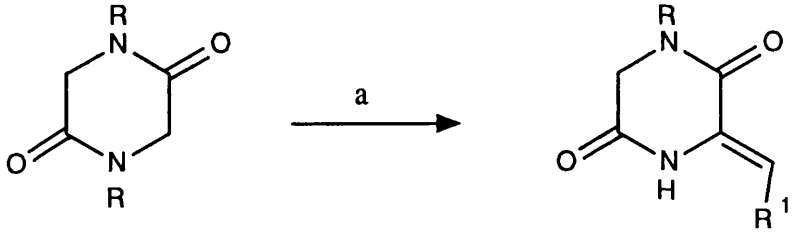
To establish the condensation of the piperazin-2,5-dione (**222**) with an aldehyde to generate the desired alkene (**223**) the piperazin-2,5-dione (**222**) had to be suitably protected and activated.



223

In 1973, Gallina *et al* [109] reported the use of the bis-acylated piperazin-2,5-dione (**224**) in their preparation of alkylidene/arylidenes (**225**) (Scheme 64).

Scheme 64



224 **225**

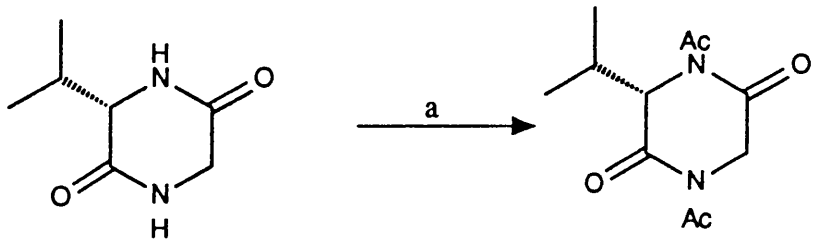
where R= Ac , R^1=Ar

Reagents : (a) i) *t* - BuOK , *t* - BuOH , 0°C ; ii) R^1CHO , DMF , 12h , RT

This procedure not only solves the problem of activation of the piperazin-2,5-dione (**222**) but provides us with suitable conditions for the condensation process to afford the benzylidene products (**223**).

The bis-acetylated piperazin-2,5-dione (**226**) was prepared by heating a suspension of the highly insoluble piperazin-2,5-dione (**222**) in acetic anhydride to 110°C until all the starting material (**222**) had dissolved. The brown residue was purified by flash chromatography to yield a pale yellow crystalline solid in 78% yield. The ^1H NMR spectrum clearly showed that the desired product (**226**) had been formed (Scheme 65).

Scheme 65



222 **226**

Reagents : (a) Ac_2O , 110^0C

Despite the severe conditions no evidence of racemisation of the L-valine residue was detected. This was also verified by Kanmera *et al* [110] in a similar cyclo (L-Ala-L-Gly) system. Unfortunately under these conditions polymerisation is significant. So other acylation techniques [333] were tried. One of the major hurdles to overcome in the acylation of piperazin-2,5-diones was their insolubility in many polar solvents.

The acylation step was repeated using DMF as solvent, while maintaining acetic anhydride as the acylating source. After refluxing for 4h the solution was worked up

and purified in the usual manner to yield **226** in 45% yield.

The introduction of a base was then considered. Pyridine was added to a suspension of **222** in acetic anhydride . This was brought to reflux for 7h and after purification yielded **226** in a reasonable yield (57%). A repeat of the latter step using ultrasonification instead of heat gave poor yields of **226** (29%).

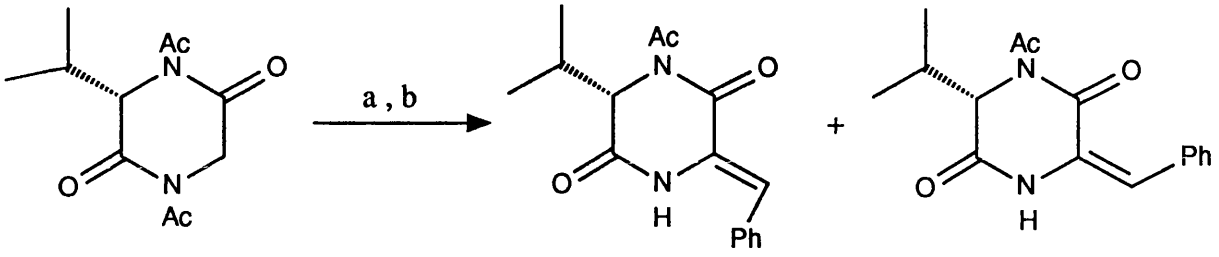
The replacement of the acylating source was then employed. Acetyl chloride was added to a suspension of **222** in DMF. After refluxing for 6h purification of the residue gave **226** in only 43% yield.

The generation of the more reactive ketene, *in situ*, in the presence of the reacting substrate has been reported [334] by the treatment of acetyl chloride with a tertiary amine. This was considered a viable method of imide formation. The treatment of the piperazin-2,5-dione with DBU in the presence of acetyl chloride at room temperature, however, gave poor yields of the desired product (**226**) (33%). Again polymerisation was suspected as the main source of trouble.

*Condensation of the bis-acetylated piperazin-2,5-dione (**226**) to afford the benzylidene (**227**)*

Condensation of the bis-acetylated piperazin-2,5-dione (**226**) was achieved by the procedure described by Gallina *et al* [109]. The treatment of **226** with a solution of potassium-*t*-butoxide in THF in the presence of benzaldehyde gave mainly the monoacetylated (Z)-benzylidene (**227Z**) (Z:E, 8:1) in good yield (83%). The two geometrical isomers (**227Z/E**) were separated by flash chromatography (Scheme 66).

Scheme 66



226 **277Z** **227E**

Reagents : (a) PhCHO , DMF ; (b) *t* - BuOK , THF , 0°C, 4 h

The ^{1}H NMR spectra of both alkenes (**227Z** and **227E**) showed the presence of a phenyl multiplet and an alkene singlet indicating the addition-elimination had been successful. It also showed that one of the acetyl groups had been removed in the process. The overall structure of **227Z** and **227E** were proved by difference n.O.e. experiments.

In the case of **227Z** the N-H signal (δ 8.0 ppm) was enhanced when the phenyl group (δ 7.44 ppm) was irradiated whilst no N-H enhancement was observed when the vinyl proton (δ 7.15 ppm) was irradiated. Thus indicating, a (Z)-stereochemistry about the C=C bond.

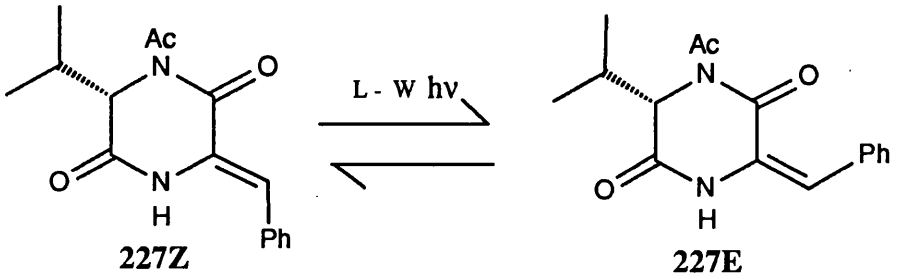
For compound **227E**, however, only the vinyl proton was enhanced (δ 6.65 ppm) when the N-H signal (δ 9.65 ppm) was irradiated. Thus indicating an (E)-stereochemistry about the C=C bond.

The major product was thus confirmed as the kinetic and more thermodynamically stable Z-isomer (**227Z**) arriving through the β-elimination of acetic acid after an N-Ac to O-Ac transfer.

Photoequilibration of the (Z)-benzylidene (**227Z**) *into its (E)-geometrical isomer* (**227E**).

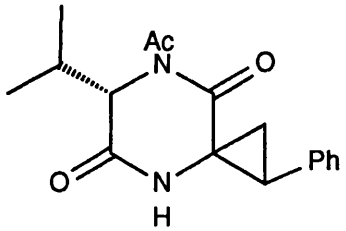
It was noticed that when the (Z)-benzylidene (**227Z**) was left for any period of time in daylight that it partially converted into its isomeric (E)-form (**227E**) *i.e.* **227Z** and **227E** were in photoequilibrium (Scheme 67).

Scheme 67



227Z **227E**

This has been reported in other unsaturated systems [335]. The irradiation of a solution of **227Z** in chloroform with L-W U.V. light (λ =370nm) for 24h reaffirmed this observation in converting 35% of the original (Z)-component into its (E)-form. Thus, here lies a technique for the generation of the (E)-isomer which allows access to the synthesis of (E)-2,3-methanophenylalanine (**228E**).



228E

Cyclopropane construction

There are three major techniques which can be employed in the preparation of a cyclopropane from a α,β-unsaturated amide:-

(i) the Simmons-Smith reaction

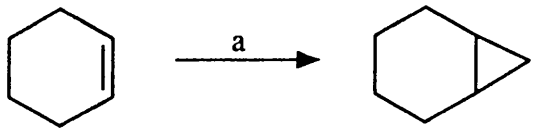
(ii) the addition of a diazoalkane

and (iii) the addition of a sulphoxonium ylide

Simmons-Smith reaction

The Simmons-Smith reaction is a method of preparation of cyclopropanes from alkenes without the isolation of insertion side-products typical of free carbene intermediate processes [336]. This procedure involves the treatment of C=C containing compounds with methylene diiodide and a Zn/Cu couple leading to cyclopropanes in good yields [337] (Scheme 68).

Scheme 68

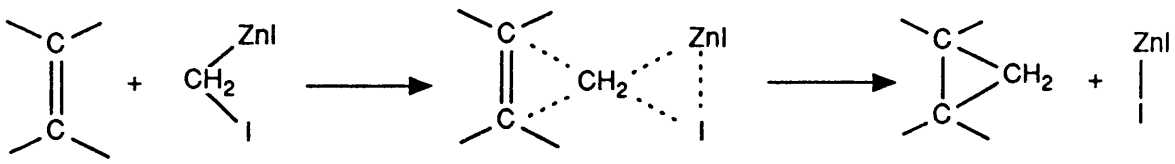


Reagents : (a) CH_2I_2 , Zn(Cu) , Et_2O , Δ

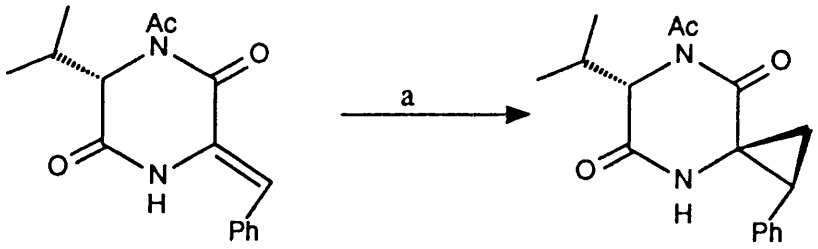
The Zn/Cu couple can be prepared in several ways [338], of which heating zinc dust with copper(I)chloride in ether under nitrogen [339] is particularly convenient. The actual attacking species is an organozinc intermediate and thought to be $(ICH_2)_2$ $Zn.ZnI_2$. The addition of this intermediate to C=C bond is stereospecifically *syn* and is thought to go *via* a concerted mechanism [340] (Scheme 69).

The Simmons-Smith conditions described by Simmons, Mash *et al* [341,342] were applied to our (Z)-benzylidene (**227Z**) having previously prepared the Zn-Cu couple by the literature procedure [338a] (Scheme 70).

Scheme 69



Scheme 70



227Z **228Z**

Reagents : (a) CH_2I_2 , Zn (Cu) , Et_2O , Δ

No carbene insertion resulted, with the majority of the starting material (**227Z**) being recovered (95%). The reason was thought to be the overall deactivation of the alkene by the electron withdrawing ester function, since Simmons and Smith reported low yielding in α,β-unsaturated esters containing β-substituted electron withdrawing groups [341].

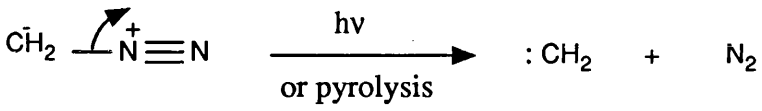
More recently a modified Simmons-Smith procedure using a Zn-Ag couple has been established [343]. This method when applied to the benzylidine (**227Z**) gave no desired product (**228Z**).

Addition of a diazoalkane

Diazomethane is an important method for the generation of cyclopropanes from alkenes. If the free carbene is required the diazomethane is initially photolysed [344]

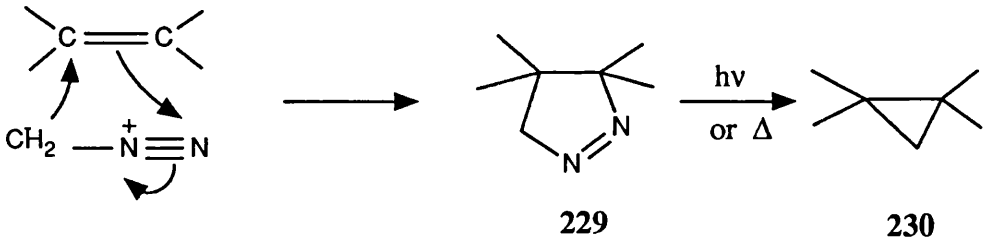
or pyrolysed [345] before the addition of the alkene substrate (Scheme 71) otherwise it is added directly.

Scheme 71



Normally direct addition of diazomethane to an alkene results in the formation of a pyrazoline intermediate (**229**) probably *via* a 2+3 cycloaddition mechanism (Scheme 72).

Scheme 72

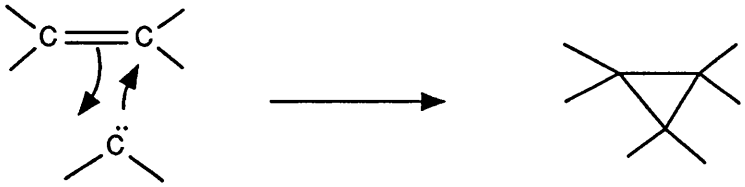


229 **230**

Often these intermediates are stable and can be isolated [249, 253] or require photolysis [344] or pyrolysis [345] for their conversion into the cyclopropane derivatives (**230**).

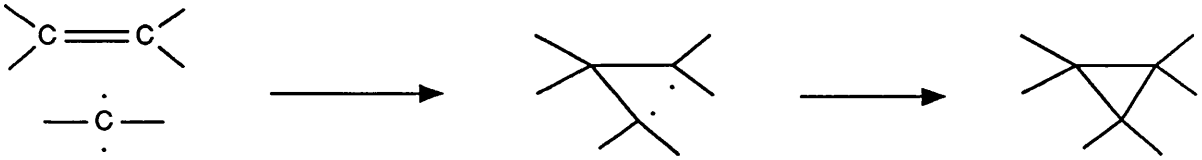
If the free carbene is generated it is usually formed as a high energy singlet species and is so reactive that it reacts before it has chance to decay into the triplet state [346]. As a consequence the reaction is stereospecific and *syn* products are formed [347] probably *via* one step mechanism [348] (Scheme 73).

Scheme 73



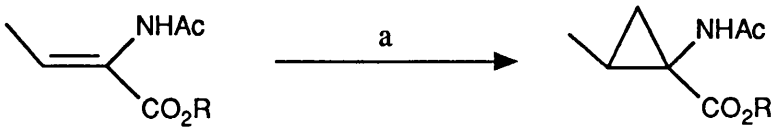
Whereas for carbenes generated in the lower energy triplet state [349] have been found to react non-stereospecifically with alkenes [350] probably by a diradical mechanism (Scheme 74).

Scheme 74



A disadvantage in the use of diazomethane is its tendency to insert into other functionalities, if present, such as oxygen *e.g.* carbonyl group, and nitrogen *e.g.* amine or amide groups. Although it has been shown [351] that diazomethane reacts preferentially towards carbon-carbon double bonds when both alkenes and amides, in conjugation, are present within a molecule. Thus the cyclopropane product (**232**) was generated without the formation of any N-methylation product (Scheme 75).

Scheme 75



231 **232**

Reagents : (a) CH_2N_2 , ether

The reaction was thus attempted with our intermediates. The diazomethane was generated [352] by the treatment of N-nitroso-N-methyl-*p*-methyltoluenesulphonamide with KOH (Scheme 76).

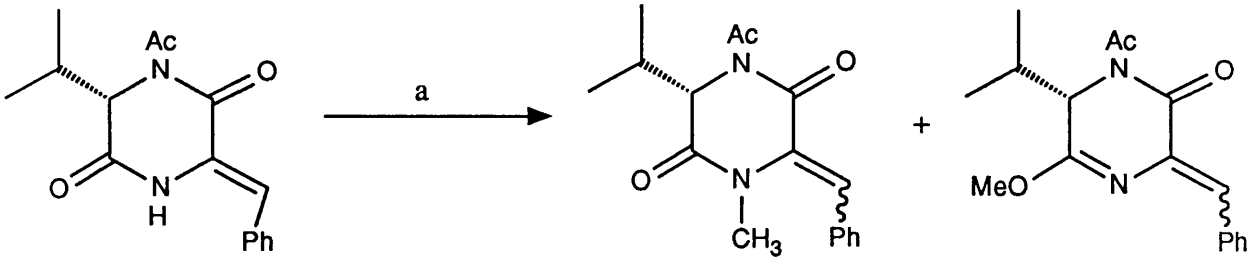
Scheme 76

Reagents : (a) KOH , H_2O , Et_2O , 70 - 75°C

In the presence of light

On the addition of 1.25 mole equivalents of diazomethane to the (Z)-benzylidene (**227Z**) at room temperature two major products were formed, neither of which corresponded to the desired cyclopropane (**228Z**) (Scheme 77).

Scheme 77

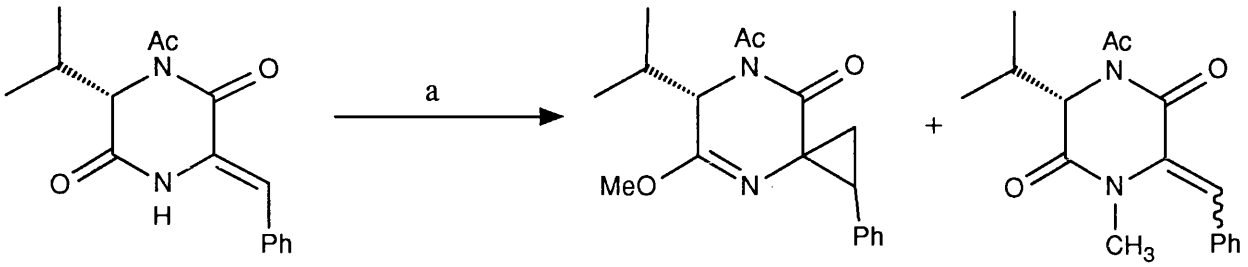


227Z **233** **234**

Reagents : (a) CH_2N_2 , ether , room temp . , 1d

These compounds, **233** and **234**, occurred as a result of N- and O-methylation, respectively. The reaction was then repeated using an excess of diazomethane. It was found that on allowing the solution to stir at room temperature for 2 days the desired cyclopropane (**235**) was formed as a mix of two diastereomers (4:1) together with the N-methylated product (**233**) (Scheme 78).

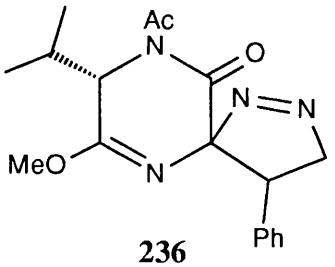
Scheme 78



227Z **235** **233**

Reagents : (a) CH_2N_2 (10 mol eq.) , ether , RT , 2d

It is interesting to note that no pyrazoline intermediate (**236**) was isolated as a consequence of alkene insertion of diazomethane.

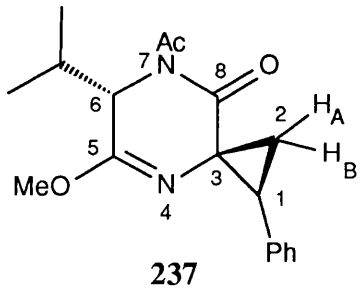


236

This indicated the intermediate (**236**) was sufficiently unstable to collapse directly to the cyclopropane without the need of photolytic or pyrolytic techniques.

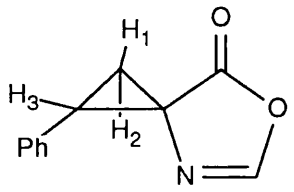
The two cyclopropyl diastereomers were separable by flash chromatography to give the major compound (**237**) as a white solid in 30% yield.

The absolute stereochemistry of **237** was determined by NMR studies. The ^1H NMR spectra indicated an ABX system for the cyclopropyl protons [δ 1.89 (1H, dd, $J_{2,2}$ 4.8, $J_{2B,1}$ 8.4, 2B-H), 2.38 (1H, dd, $J_{2,2}$ 4.8 $J_{2A,1}$ 9.9, 2B-H), 2.88 (1H, dd, $J_{1,2B}$ 8.4, $J_{1,2A}$ 9.9, 1-H)].



237

This is in following with the proposed structure since the coupling constants (J) agree with the ruling that $J_{syn}>J_{anti}>J_{gem}$. The chemical shifts and coupling constants were found to be similar to that of a (Z)-phenyl substituted spirocyclopropane (**238**) [173].



238

The δ and J values are given below in Table 9.

Table 9

Z-isomer	
δ_H(ppm)	J(Hz)
δ_1 2.38	J_{12}-5.5
δ_2 2.24	J_{23} 8.7
δ_3 3.20	J_{13} 9.7

A crystal structure of the N-tosyl derivative of (2S,3S)-2,3-methanophenylalanine later derived from the (Z)-cyclopropane (**237**) concluded that the proposed structure was indeed correct (See Appendix for X-ray structure).

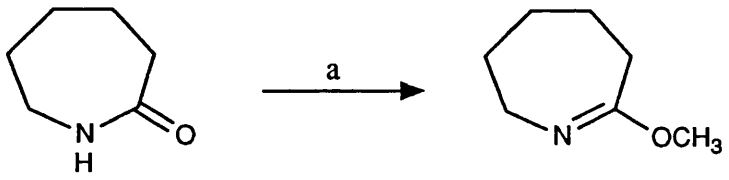
Catalysed carbene transfer

Methods were now sought to improve the diastereoselectivity of the carbene transfer and to improve the overall yield of cyclopropane. The addition of diazomethane to the benzylidene (**227Z**) was repeated in the absence of light but gave a similar ratio of diasteromers (**235**) (3:1) with the (1S,3S)-configuration as the major diasteromer, as before.

Use of silica template

In 1979, Ohno *et al* reported the synthesis of the caprolactim (**240**) from the caprolactam (**239**) *via* treatment with diazomethane in the presence of a silica gel catalyst [353] (Scheme 79).

Scheme 79



239 **240**

Reagents : (a) CH_2N_2 , ether , silica gel , $NaHCO_3$

The use of a silica gel template to bind the substrate was considered a plausible method to obtain selective cyclopropanation once the alkene (**227Z**) had been bound to the template. On treatment with diazomethane, however, no desired O-methylated cyclopropane was obtained.

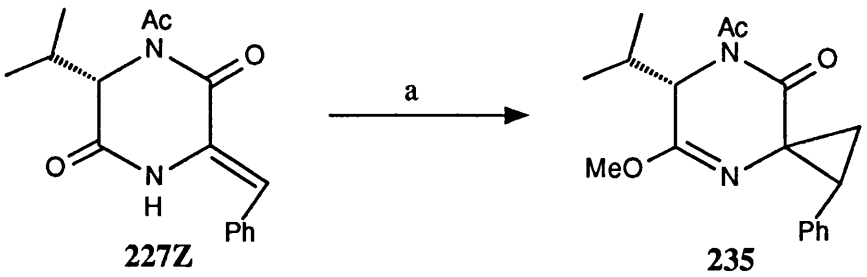
Use of transition metal complexes

The generation of cyclopropanes *via* catalytic transfomration of diazo compounds with olefins is generally attributed to occur by the interaction of an electrophilic metal

carbene with an olefin. However the specific details of that interaction remains unresolved [354]. A broad selection of catalysts for cyclopropanation have been identified. Originally copper complexes were the main source of catalysis within this field [355], but more recently this has been strongly challenged by some group VIII metals *e.g.* palladium, rhodium, *etc.* [356].

Initially palladium (II) acetate was selected as a suitable catalyst to use after claims of near quantitative cyclopropanation were reported for α,β-unsaturated ketones on treatment with catalysed diazomethane [357]. The addition of an ethereal solution of diazomethane to a suspension of the benzylidene (**227Z**) and palladium (II) acetate in ether afforded, after work-up, a 3.5:1 mix of the O-methylated cyclopropanes (**235**) in 27% yield (Scheme 80).

Scheme 80



227Z **235**

Reagents : (a) CH_2N_2 , $Pd(OAc)_2$ cat ., ether

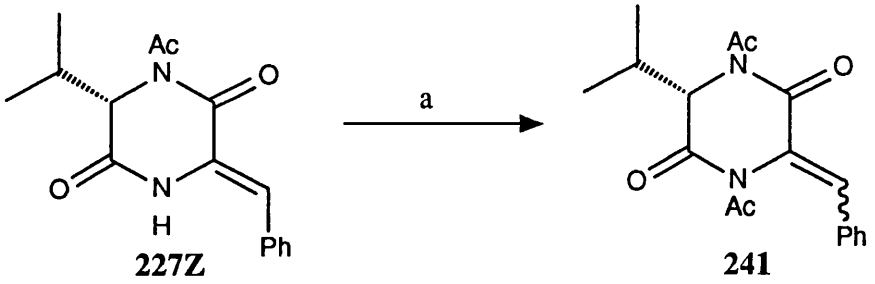
The use of palladium (II) chloride gave similar diastereoselectivity (4:1) and yield (23%). In both cases ^1H NMR studies indicated the major diastereomer to have the same configuration as for the uncatalysed diazomethane reaction *i.e.* the (Z)-(1S,3S)-configuration.

The low yields obtained during the cyclopropane formation, from the alkene (**227Z**), clearly indicated that the amide function would have to be protected as to inhibit the

competitive N- and O-methylation during carbene transfer. Acylation seemed the natural choice since by using the same amide protecting group, as already present, deprotection at a later stage would be simplified.

Acylation was achieved by heating the (Z)-benzylidene (**227Z**) in acetic anhydride in the presence of pyridine affording the bisacylated product (**241**) as a white solid (52%) (Scheme 81).

Scheme 81

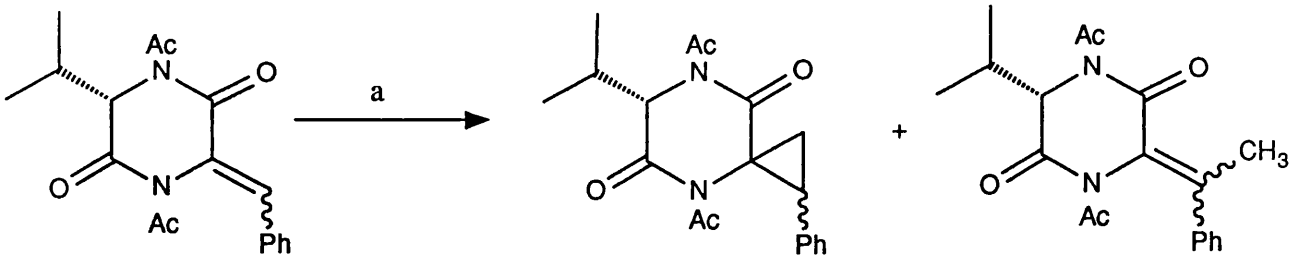


227Z **241**

Reagents : (a) Ac_2O , pyridine , 70°C , 7h

The treatment of **241** with an excess of ethereal diazomethane, in daylight, afforded an mix of the desired cyclopropane (**242**) and the vinyl methyl adduct (**243**) (1:1.5) in 13% yield. These were found to be inseparable by flash chromatography (Scheme 82).

Scheme 82

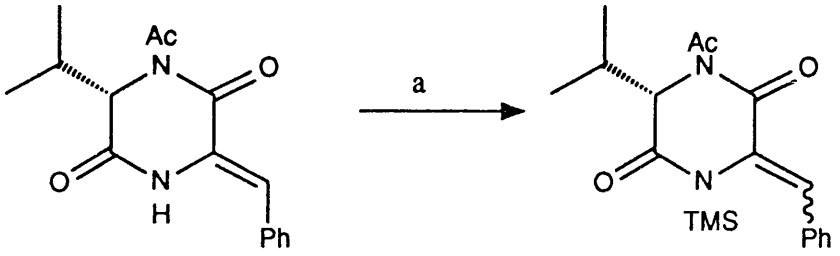


241 **242** **243**

Reagents : (a) CH_2N_2 , ether , RT

The protection of the amide nitrogen in the alkene (**227Z**) with a silyl group was then attempted. The treatment of **227Z** with trimethylsilyl chloride and base gave the desired products (**244E/Z**) as a mix of (Z)- and (E)- isomers (Scheme 83).

Scheme 83



227Z **244E/Z**

Reagents : (a) i) *t* - BuOK , THF , 0°C , ii) TMS - Cl

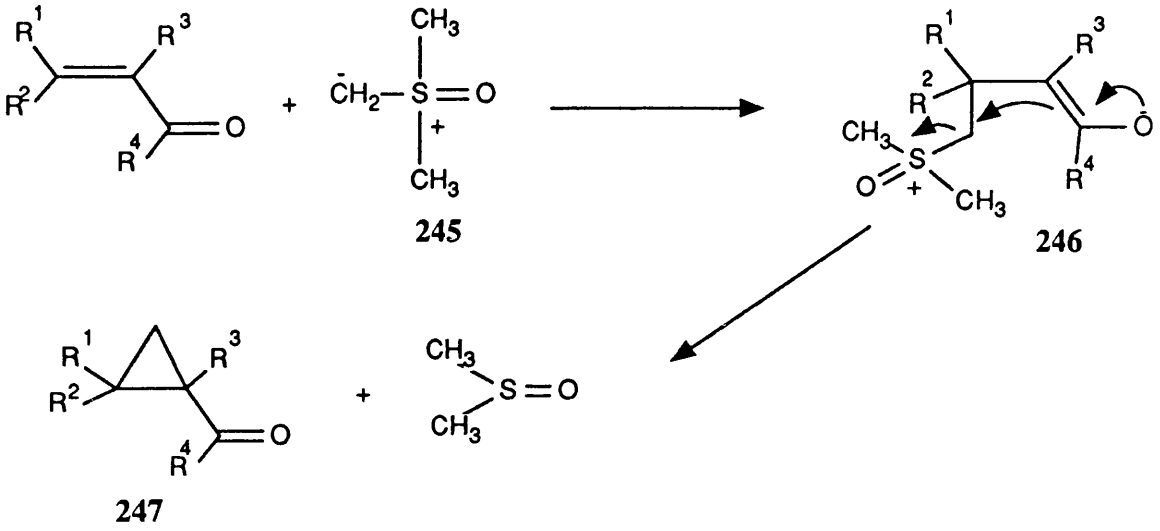
Separation of the geometrical isomers of **244E/Z** *via* flash chromatography unfortunately resulted in deprotection to yield the starting material (**227E/Z**).

The next strategy for cyclopropane construction was *via* the addition of a sulphoxonium ylide to the bis-acylated alkene (**241**).

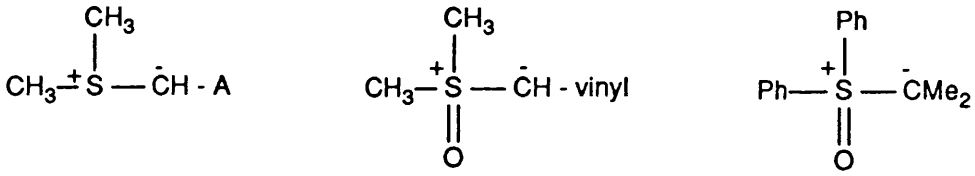
Addition of a sulphoxonium ylide

In 1965, Corey and Chaykovsky showed that dimethylsulphoxonium ylide (**245**) reacts with unsaturated conjugated ketones by a Michael addition to give cyclopropyl ketones (**247**) [358]. A two step mechanism involving the dipolar intermediate (**246**) and subsequent elimination of dimethylsulphoxide seems best to explain the course of the reaction (Scheme 84).

Scheme 84

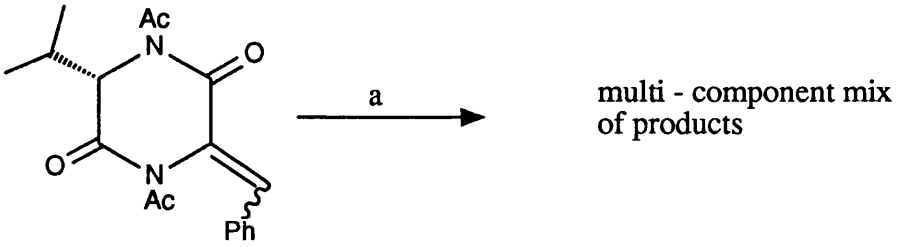


Since then other sulphur ylides *e.g.* **248** (A=acyl [359], carbethoxy [360], **249** [361], and **250** [362], which transfer CHA, CH-vinyl, and CMe_2 respectively, have been used.



The addition of a sulphoxonium ylide was then applied to our system. The dimethylsulphoxonium ylide was prepared following Trosts procedure [363]. To this was added a solution of the bisacylated benzylidene (**241**) in DMF. After the application of heat and work-up a ^1H NMR study of the residue revealed a multicomponent mix of products together with unreacted starting material (Scheme 85).

Scheme 85



241

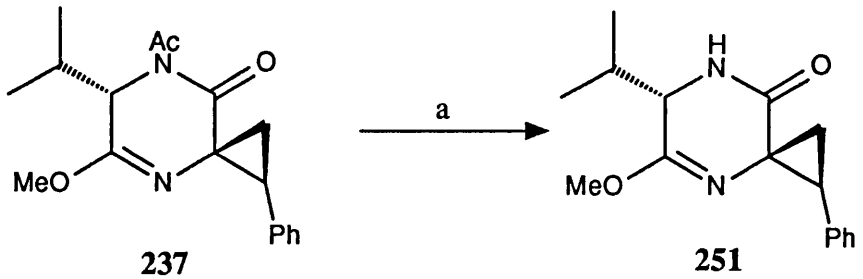
Reagents : (a) $^{-}CH_2\overset{+}{S}(O)Me_2$, DMF, 0 - 100°C

Overall the direct use of diazomethane with the benzyldine (**227Z**) was found to give the best results. Despite the low yield of cyclopropane (**235**) a 4:1 diastereoselectivity was obtained of which a single diastereomer could be obtained in pure form. In addition, the cyclopropane (**235**) contained an imine ether moiety as a result of O-methylation. This is fortunate since the amide bonds will later require suitable modification to allow hydrolysis to be achieved, and provided access to the preparation of the ∇Phe-Val dipeptides.

Deprotection of the cyclopropane (**237**)

The deprotection of **237** was achieved quite cleanly by the treatment with potassium carbonate in methanol at room temperature to give the desired amide (**251**) in quantitative yield (Scheme 86).

Scheme 86



237 **251**

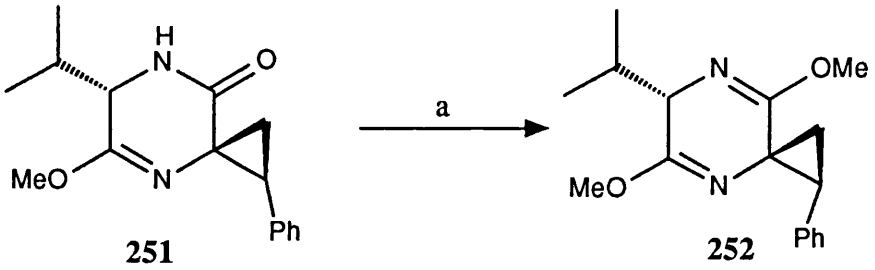
Reagents : (a) K_2CO_3 , MeOH , RT , 15 mins

Preparation of (Z)-(2S,3S)-2,3-methanophenylalanine methyl ester (**253**).

The procedures of Schöllkopf [332] were employed in the conversion of the amide (**251**) to the desired 2,3-methanophenylalanine methyl ester (**253**).

To a solution of the amide (**251**) in DCM was added trimethyloxoniumtetrafluoroborate. After two days, work-up yielded the desired bislactim ether (**252**) as a colourless oil in 71% yield (Scheme 87).

Scheme 87

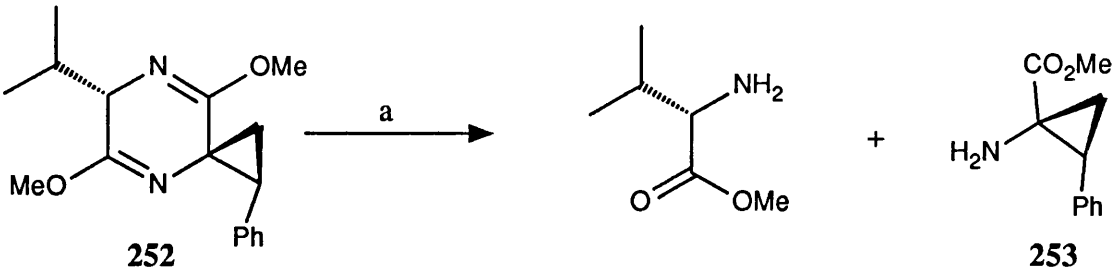


251 **252**

Reagents : (a) Me_3OBF_4 ,DCM ,RT , 2d

The hydrolysis of the bislactim ether (**252**) under mild acidic conditions afforded, after work-up, a mixture of the desired product (**253**) together with L-valine methyl ester (Scheme 88).

Scheme 88



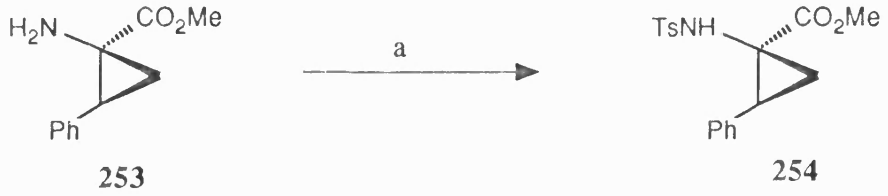
252 **253**

Reagents : (a) i) 0.25M HCl ,ether , RT , 24h , ii) NH_3(aq) , pH9 , 0^0C

These were found to be easily separated *via* flash chromatography on silica gel to give the 2,3-methanophenylalanine methyl ester (**253**) in 58% yield as a colourless oil.

The absolute stereochemistry of **253** had yet to be proven. The treatment of 2,3-methanophenylalanine with triethylamine and tosyl chloride generated the N-tosyl derivatives (**254**) in 78% yield as a colourless solid (Scheme 89).

Scheme 89



253 **254**

Reagents : (a) TSCl , Et_3N , THF , Δ , 2 h

Recrystallisation of (**254**) from ethyl acetate-petrol provided crystals suitable for an X-ray crystallographic determination, which duly confirmed our assignment of this structure as (Z)-(2S,3S)-2,3-methanophenylalanine methyl ester[(Figure 4) full details are in Appendix)].

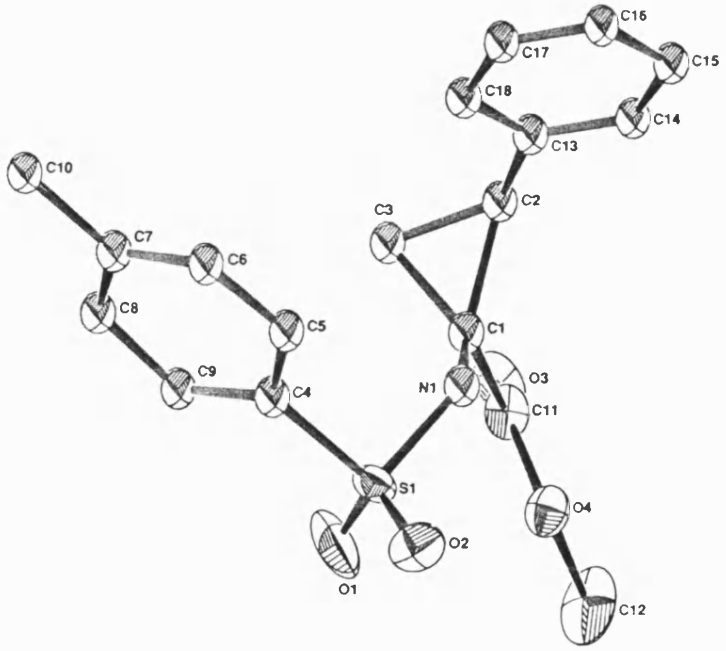


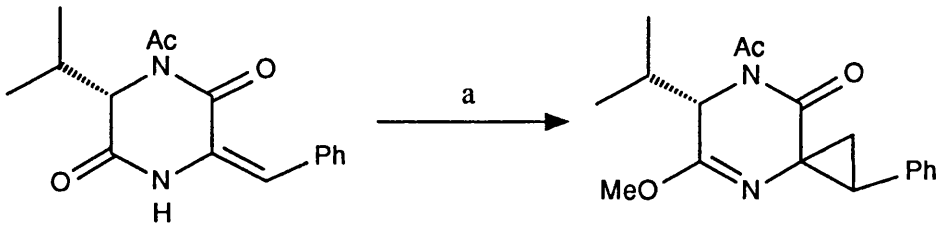
Fig 4. X-ray plot of (2S,3S)-N-tosylamino-∇^{Z}Phe-OMe(ORTEP plot).

Having successfully found a diastereomeric synthesis for (Z)-2,3-methano-phenylalanine methyl ester (**253**), their (E)-analogues were then prepared following the same synthetic pathway.

Preparation of (E)-2,3-methanophenylalanine methyl ester (**259**)

The (E)-benzylidene (**227E**), prepared through photoequilibration of the (Z)-isomer (**227Z**), was treated with ethereal diazomethane to afford the desired cyclopropane (**255**) as a mix of two diastereomers (4:1) (Scheme 90).

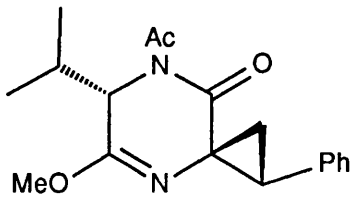
Scheme 90



227E **255**

Reagents : (a) CH_2N_2 , ether , RT , 2d

The major isomer (**256**) was separated by flash chromatography and isolated as a colourless oil in 14% yield.

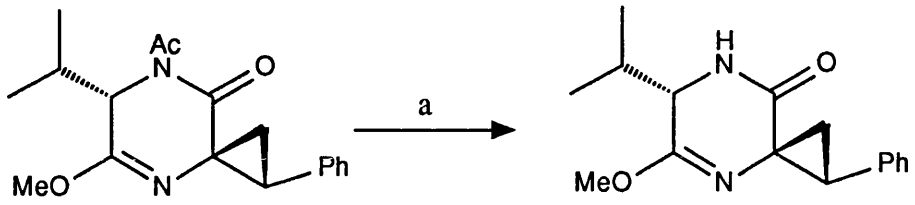


256

Deprotection of the cyclopropane (**256**)

The desired amide (**257**) was prepared uneventfully by treating the cyclopropane (**256**) with potassium carbonate in DCM as a colourless oil in quantitative yield (Scheme 91)

Scheme 91



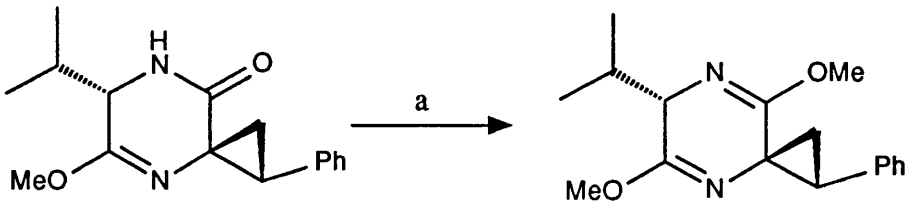
256 **257**

Reagents : (a) K_2CO_3 , DCM , RT , 2h

Preparation of the (E)-(2S,3R)-2,3-methanophenylalanine methyl ester (**258**).

The treatment of the amide (**257**) with Meerwein's reagent gave the (E)-bislactim ether (**258**) in 65% yield albeit a lower yield than the (Z)-isomer (**252**) (Scheme 92)

Scheme 92



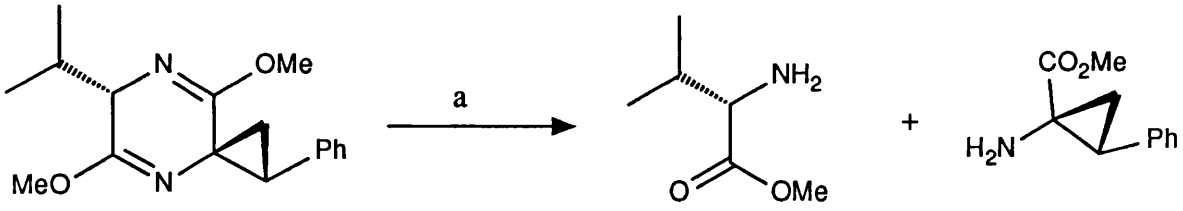
257 **258**

Reagents : (a) Me_3OBF_4 , DCM , RT , 2d

The hydrolysis of (**258**) under mild acidic conditions gave a mixture of L-valine methyl ester and (E)-2,3-phenylalanine methyl ester (**259**) after basification. (Scheme 93).

These were readily separated by flash chromatography on silica gel to afford the desired (E)-2,3-methanophenylalanine (**259**) in low yield (22%).

Scheme 93



258 **259**

Reagents : (a) i) 0.25M HCl , ether , RT , 24h , ii) 2M NH_4OH , pH6 , 0°C

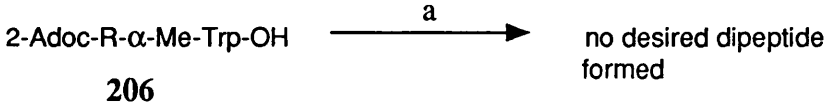
2.4 Preparation of 2,3-methanophenylalanine dipeptide derivatives

Coupling of (Z)- and (E)-2,3-methanophenylalanine ester (253 and 259) with an N-protected tryptophan (206)

Preparation of the (Z)-dipeptide ester (**260**)

The coupling conditions used for the preparation of the dehydrophenylalanine dipeptide series (Section 1.3) were initially employed. The N-protected tryptophan (**206**) was initially activated by the addition of HOBt/DCC and to this was added (Z)-amino ester (**253**) at room temperature. After work up only unreacted starting material was recovered in 80% yield (Scheme 94).

Scheme 94



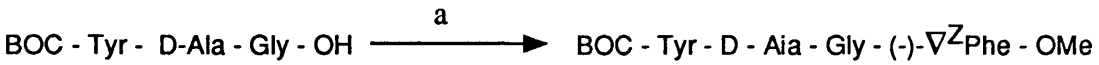
206

Reagents : (a) i) HOBT , DCC , EtOAc , ii) (2S,3S)-∇^{Z}Phe-OMe , EtOAc , RT

Clearly the activated ester generated *in situ* was insufficient to favour attack by the hindered amine of the amino ester (**253**). It was noticed that Stammer *et al* had previously reported [364] the preparation of peptides containing (Z)-2,3-methano-phenylalanine by using a mixed anhydride approach in their synthesis of cyclopropyl

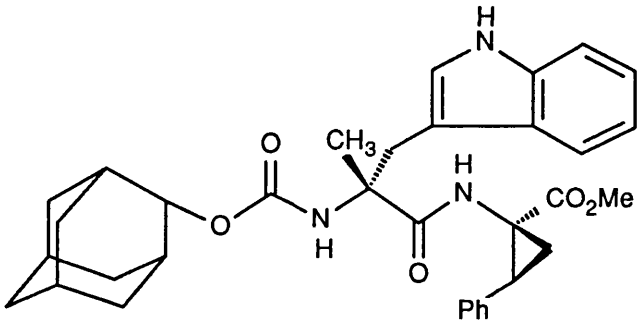
enkephalin analogues (Scheme 95).

Scheme 95



Reagents : (a) i) NMM , isobutyl chloroformate , THF , -10°C , 30 mins. ,
ii) (-)-∇^{Z}Phe - OMe . HCl , NMM , $CHCl_3$, 0°C

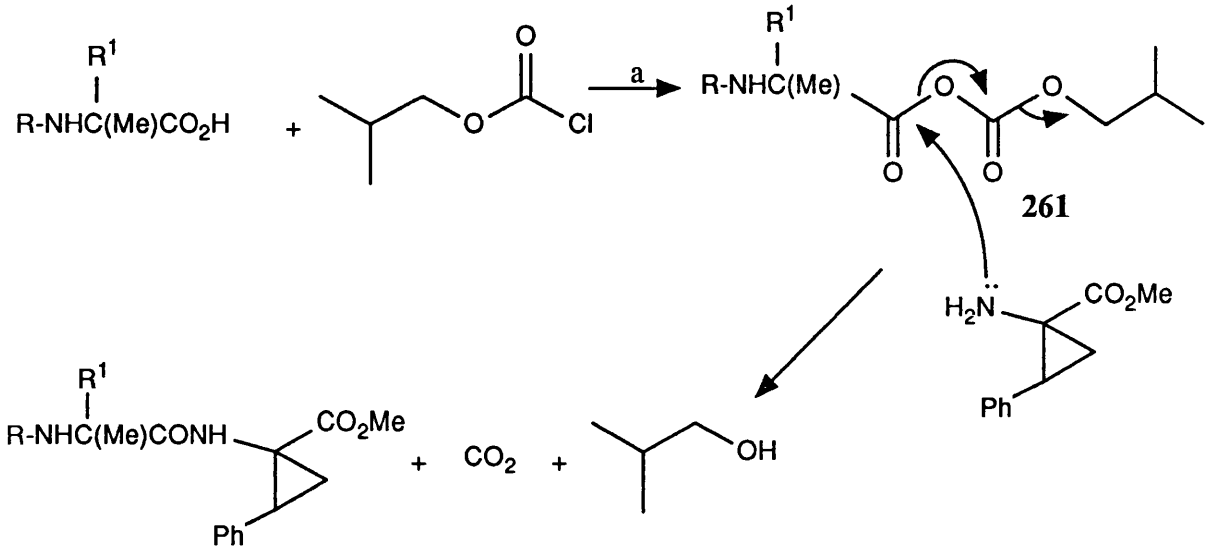
These conditions were applied to our system and afforded the desired dipeptide (**260**) as a yellow foam in 40% yield.



260

This procedure was successful owing to the anhydride (**261**) generated being highly activated towards nucleophilic attack facilitated by the loss of carbon dioxide and isobutanol (Scheme 96).

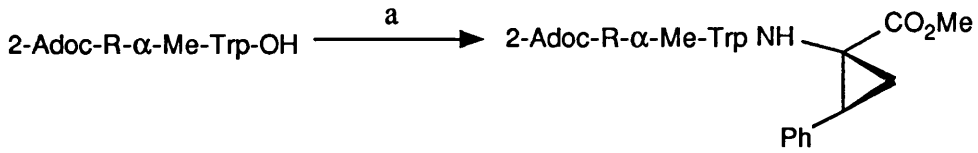
Scheme 96



Reagents : (a) NMM , THF , 0°C .

An adapted DCC coupling procedure was then tried whereby 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was used instead of DCC for ease of removal of the urea by-product (EDU) on work-up. This gave no desired dipeptide (**260**) at room temperature but under more forcing conditions **260** was achieved but in low yield (31%) (Scheme 97).

Scheme 97



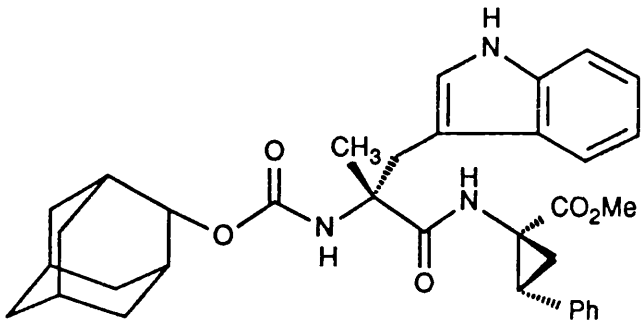
260

Reagents : (a) i) NMM , HOBT , EDC , THF , 0°C , 1h , ii) (2S,3S)∇^{Z}Phe - OMe , THF , Δ , 1d

Preparation of the (E)-dipeptide ester (**262**)

The mixed anhydride approach was applied to the coupling of the (E)-amino ester (**259**) with the N-protected tryptophan (**206**). On employing the same conditions, as for the

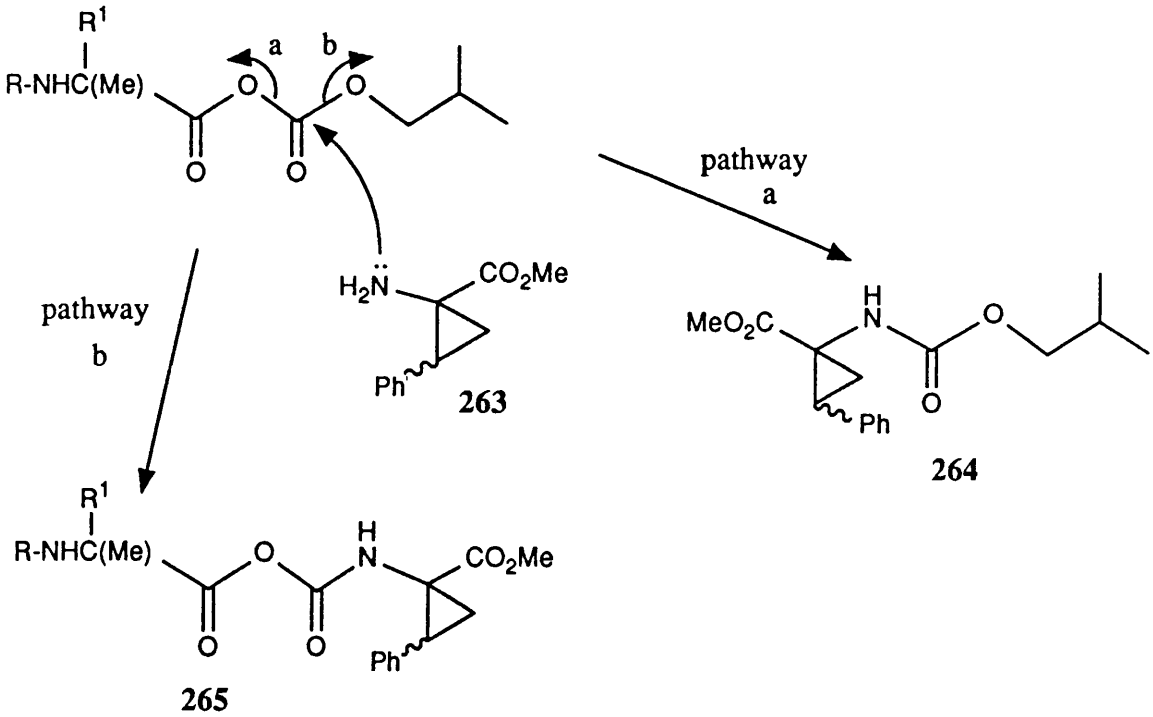
(Z)-amino ester (**253**), the desired product (**262**) was obtained but in slightly lower yield (32%).



262

It was noticed during these mixed anhydride couplings that several by-products were obtained. These were not isolated but were likely to be acyl and anhydride derivatives (**264**) and (**265**) of the starting amino esters (**263**) formed as a result of competing *iso*-butoyl carbonyl attack [365] (Scheme 98).

Scheme 98



263

264

265

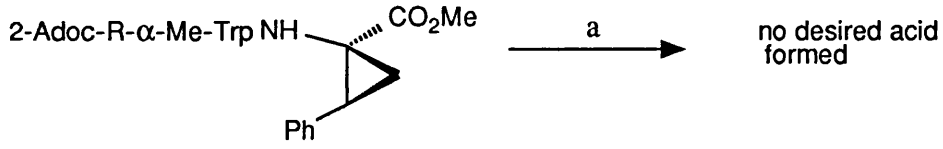
These side reactions would account for the low yield of the desired (Z)- and

(E)-dipeptide esters (**260**) and (**262**).

Hydrolysis of the dipeptide ester (**260**)

The same hydrolysis techniques were employed as for the dehydrodipeptide ester (**219Z**). The use of lithium hydroxide similarly gave no desired acid even after reflux (Scheme 99), unreacted starting material was recovered in 85% yield.

Scheme 99

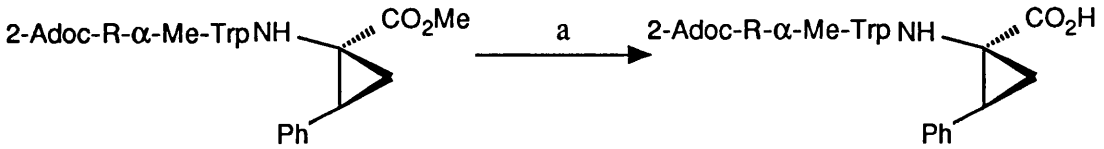


260

Reagents : (a) 0.1M LiOH (aq) , THF , Δ , 3h

The use of 0.1M sodium hydroxide with the ester (**260**) in ethanol was attempted, which after reflux for 2h afforded the desired acid (**266**) in crude form (88% yield). The product was then further purified by reverse-phase chromatography to give the acid as a white foam. The ^1H NMR of the acid (**266**) confirmed its structure (Scheme 100).

Scheme 100

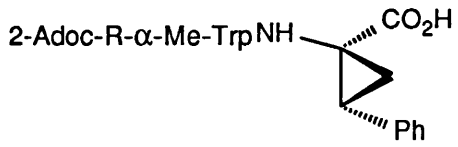


260 **266**

Reagents : (a) 0.1M NaOH (aq) , EtOH ,Δ , 3h

Insufficient material of the (E)-dipeptide ester (**262**) and time prevented the

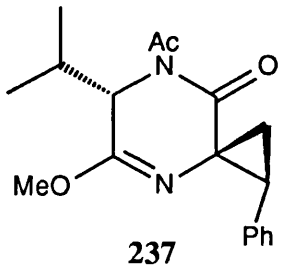
corresponding acid (**267**) from being made by these techniques.



267

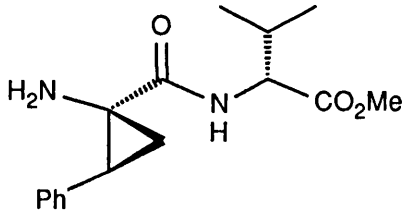
Attempted hydrolysis of the imine ether (**237**)

As a result of treatment of the (Z)-benzylidene (**227Z**) with an excess of diazomethane it was found that methylene not only inserted into the carbon-carbon double bond to form the cyclopropane, as desired, but also added into the amide carbonyl oxygen to give the imine ether adduct (**237**) (See Section 2.3).



237

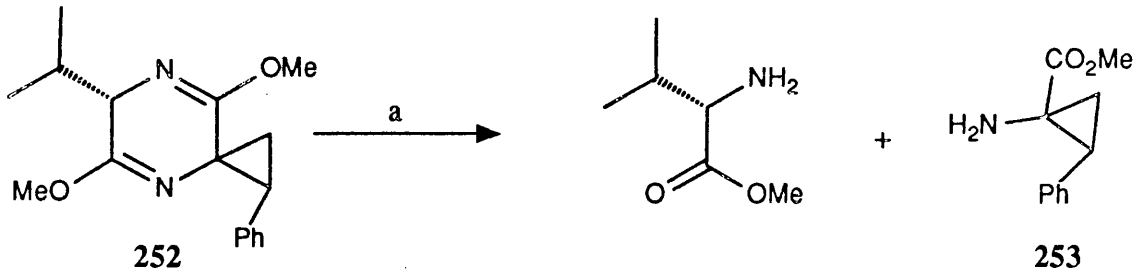
Thus, selective hydrolysis of the more reactive imine bond would give access to the (Z)-2,3-methanophenylalanyl-L-valine dipeptides (**268**).



268

The obvious choice of hydrolysis reagent was mild acidic conditions since this was successfully employed in the hydrolysis of the bismine ether (**252**) to afford the 2,3-methanophenylalanine methyl ester (**253**) (Scheme 101).

Scheme 101

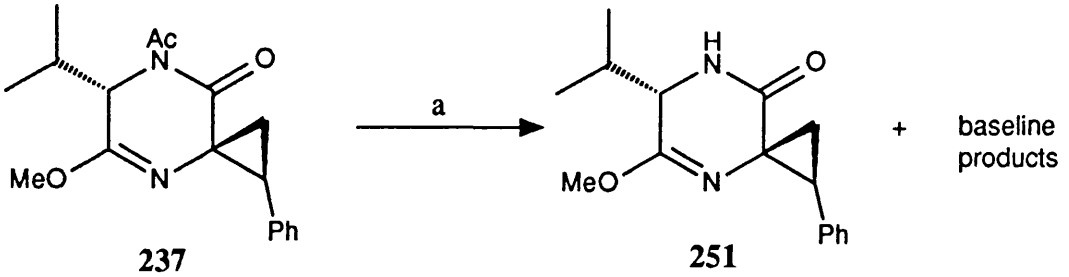


252 **253**

Reagents : (a) i) 0.25M HCl , ether , RT, 24h , ii) NH_3(aq) , pH9 , 0°C

These conditions were applied to the imine ether (**237**). After 2h at room temperature normal-phase tlc indicated that all the starting material had been transformed into the deacylated product (**251**) together with baseline products (Scheme 102).

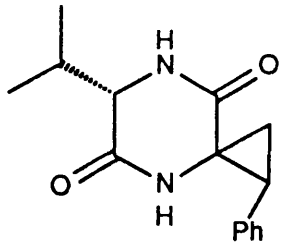
Scheme 102



237 **251**

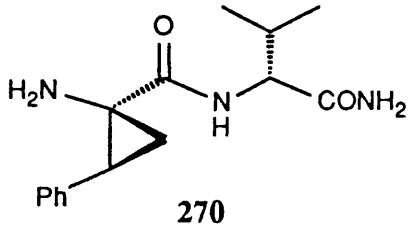
Reagents : (a) 0.25M HCl , ether , RT, 2d

After work-up ^{1}H NMR of the organic phase verified that the deacylated product (**251**) had been formed. Reverse-phase tlc on the remaining aqueous phase indicated an unseparable mix of components. One of which is likely to be the stable piperazin-2,5-dione (**269**).



269

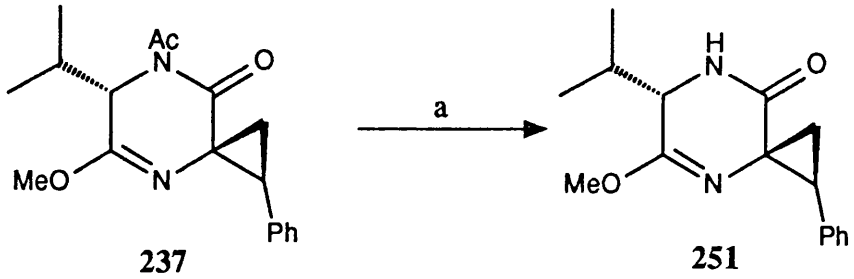
Clearly hydrolysis of the imine ether was being achieved, however, the hydrolysed product (**268**) was recyclising and/or undergoing further hydrolysis. To overcome these problems hydrolysis using an ammonium hydroxide source was considered a good alternative since the amide (**270**) formed upon hydrolysis would be more stable to recyclisation than the corresponding ester.



270

An aqueous ammonia solution was added to the imine ether (**237**) at room temperature and the reaction was monitored by tlc. No baseline products were evident after 2 days. ^{1}H NMR revealed only partial conversion to the deacylated product (**251**) (10% yield) (Scheme 103).

Scheme 103

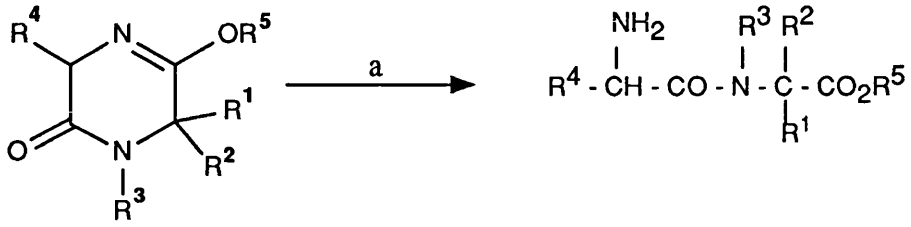


237 **251**

Reagents : (a) 33% w/w NH_3 (aq) , THF , RT , 2d

In 1990 Schöllkopf reported [366] the isolation of a dipeptide ester (**272**) *via* the hydrolysis of an imine ether (**271**) in an analogous system (Scheme 104).

Scheme 104



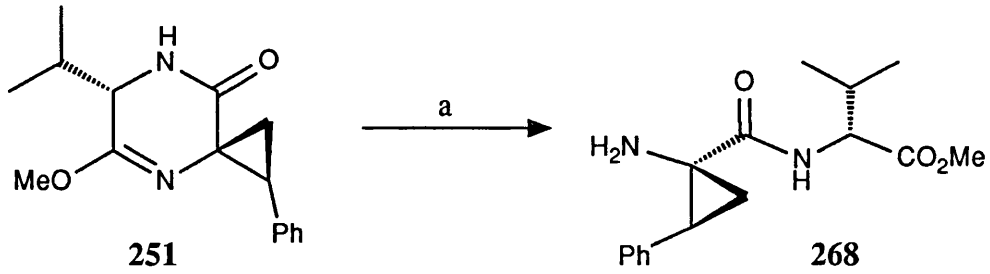
271 **272**

R^1= 1-8C alkyl , phenyl or benzyl
R^2= 1-8C alkyl (opt. interupted by O,S or COO) , phenyl or benzyl .
R^3= H
R^2+R^3= $(CH_2)_3$, $(CH_2)_4$ or $CH_2CH{=}CHCH_2$.

Reagents : (a) 0.1N HCl , CH_3CN , RT , 24h

Using mild acidic conditions and high dilution. These conditions were applied to the deacylated imine ether (**251**) to afford the desired dipeptide ester (**268**) in 82% yield (Scheme 105).

Scheme 105



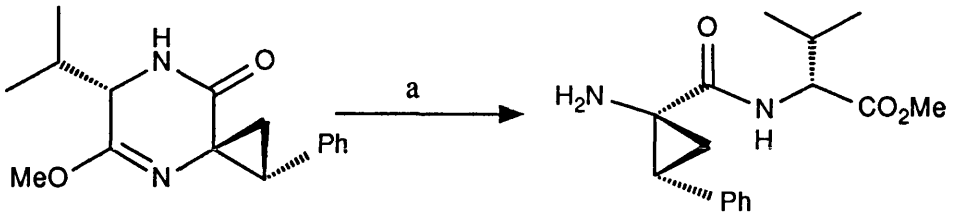
251 **268**

Reagents : (a) i) 0.1M HCl , CH_3CN , RT, 21h , ii) $NaHCO_3$ (aq) ,pH7

The compound **268** was confirmed by ^1H NMR and tlc (ninhydrin active). The same conditions were applied to the (E)-imine ether (**257**) and the (E)-2,3-methano-

phenylalanyl-L-valine dipeptide ester (**273**) was afforded in 79% yield (Scheme 106).

Scheme 106

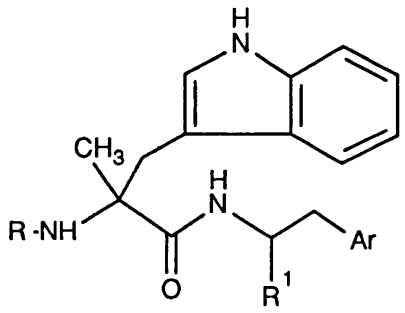


257 **273**

Reagents : (a) i) 0.1M HCl , CH_3CN , room temperature , 15h , ii) $NaHCO_3$ (aq) , pH7

2.5 Structure Activity Relationships

In 1990 Horwell *et al* reported the design of novel α-methyl-Trp dipeptide analogues of CCK (30-33) which possessed micromolar CCK-B receptor affinity [321]. For example compounds (**274A,B**) had binding affinities of 10 and 5 μMol, respectively.



274A(R=Trichloro-*t*butyloxycarbonyl (TcBoc), R^1=H, Ar= 2-Pyr)
B(R= 1-Adamantyloxycarbonyl (1-Adoc), R^1= H, Ar= Ph)

It was suggested that the minimum fragment of CCK (30-33) can be read by the receptor as the continuous message Trp-Phe, corresponding to the non-continuous message of CCK (30-33) (Trp-Met-Asp-Phe-amide) (Fig. 5).

Fine tuning of the N- and C-terminus groups of these dipeptide analogues(**274**) indicated that the C-terminus could tolerate many changes to the α-Phe side chain, e.g. (S)-Phe-$CONH_2$ (**274C**, R^1=$CONH_2$), (S)-Phe-CH_2OH (**274D**, R^1=CH_2OH) and (S)-Phe-piperidide (**274E**, R^1=CON-piperidine), without loss in binding affinity [321]. Whilst the N-terminus required bulky substituents rather than a straight chain or an aromatic hydrocarbon to achieve micromolar affinity (Table 10).

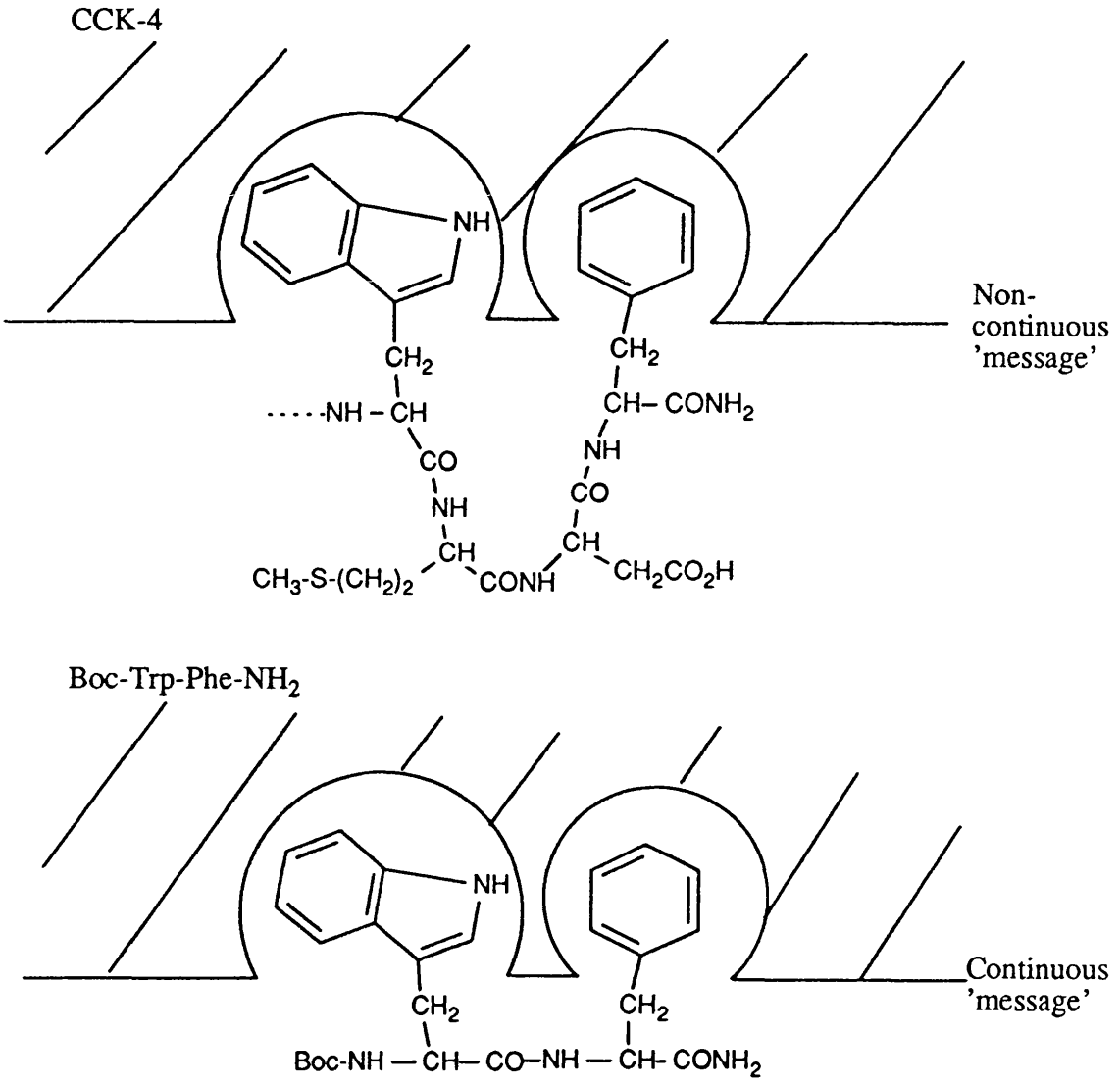
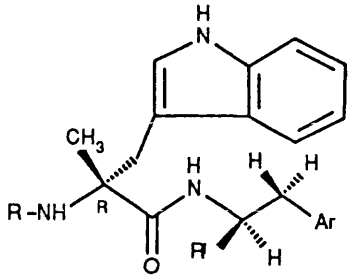


Fig.5 Model of the non-continuous Trp-Phe-message of CCK-4 mimicked by the continuous Trp-Phe-message of the dipeptide Boc-Trp-Phe-amide.

Table 10. Physical data binding affinities for α-Me-Trp-Phe and α-Me-Trp-phenethylamide. Derivatives and analogues, compounds 274A-E



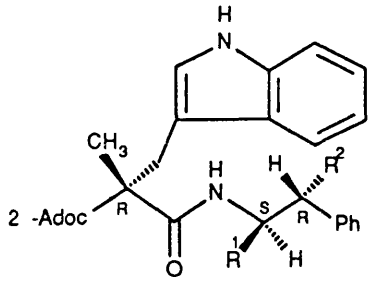
274

Entry	R	R^1	Configuration			$K_i(\mu M)$
			MeTrp	Phe	Ar	
274A	TcBoc	H	RS	-	2-Pyr	10
B	1-Adoc	H	RS	-	Ph	5
C	Boc	$CONH_2$	R	S	Ph	35
D	Amoc	CH_2OH	RS	S	Ph	9
E	Amoc	CON ⬡	RS	S	Ph	23

The 2-adamantyl group was later found to be the superior choice of N-substituent and when present in α-methyl-(R)-tryptophan derivative, for example compound **275**, Table 11, these compounds were found to have nanomolar CCK-B binding affinities [322].

In most instances these compounds possessed binding affinities comparable to CCK (30-33) itself [367].

Table 11. CCK Receptor Binding Affinities[a]

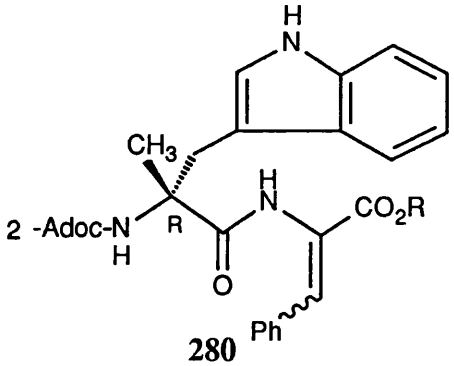


275

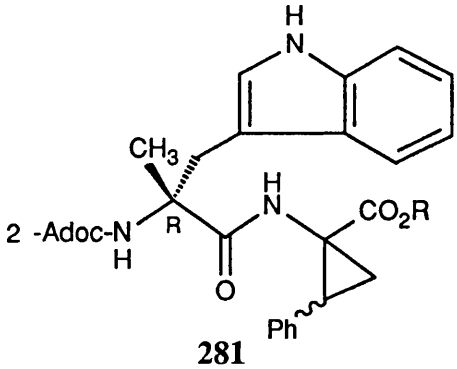
Entry	R^1	R^2	IC_{50} (nM)	
			CCK-B	CCK-A
275A	CH_2OH	H	6.3 (4.2-8.9)	780 (690-850)
275B	$CH_2OCO(CH_2)_2$-CO_2H	H	3.4 (2.5-5.8)	740 (690-790)
275C	$CH_2NHCOCH=CH$-CO_2H	H	0.8 (0.4-1.2)	440 (430-440)
275D	H	$NHCOCH=CH$-CO_2H	0.7 (0.5-1.0)	790 (680-1000)
275E	H	$NHCO(CH_2)_2$-CO_2H	1.7 (1.3-2.7)	4300 (1200-8500)
276, Devazepide (MK329)			31 (18-43)	0.1 (0.03-0.2)
277, L-365,260			5.1 (4.6-5.4)	230 (170-380)
278, CCK-8S			0.3 (0.2-0.3)	0.1 (0.08-0.2)
279, Pentagastrin			0.8 (0.5-0.9)	600 (500-660)

[a] IC_{50} represents the concentration (nM) producing half maximal inhibition of specific binding of [^{125}I] Bolton Hunter CCK-8 to CCK receptors in the mouse cerebral cortex (CCK-B) or the rat pancreas (CCK-A). The values given are the geometric mean and the range from at least three separate experiments.

This data indicates that the Trp and Phe residues are necessary to impart micromolar affinity but molecular recognition information in the Met and Asp residues of CCK (30-33) enhances affinity a further 1000-fold. Conformational energy minimisation studies of CCK(30-33) have shown a preferred helical stucture [368], in which the Trp and Phe residues are within close proximity of one another, which is in agreement with ORD data [369]. As a consequence of this, the Asp residue is readily available as an necessary binding group in which the terminal COOH group is free to explore a large volume of space [368-370]. These compounds, containing a C-terminal carboxyl group, are expected to be good candidates for CCK-4 dipeptide surrogates. If the carboxyl moiety is conformationally restricted and if its conformation, together with those of the Trp and Phe moieties, are correct to fit the receptor in the active site then, as a result of the molecules reduced degrees of freedom, the gain in entropy should cause the substrate to bind more strongly to the receptor and thus be a better agonist/ antagonist. The conformationally restricted dehydrophenylalanine and 2,3-methano-phenylalanine derivative (**280** and **281**) were seen as ideal candidates for this purpose and so were synthesised as previously described.



280



281

The binding affinities of the α-Me-(R)-Trp dipeptide derivatives prepared are given in Table 12.

Table 12 - CCK receptor binding affinities[a]

Substrate	IC_{50} (nM)	
	CCK-B	CCK-A
R,S *Threo* 2-Adoc-R-α-Me-Trp-NHC(CO_2Me)CHPh(OAc) (218)	13	-
2-Adoc-R-α-Me-Trp-Δ^ZPhe-OMe (219Z)	270	-
2-Adoc-R-α-Me-Trp-Δ^ZPhe-OH (220Z)	54	60
2-Adoc-R-α-Me-Trp-(2S,3S)-∇^ZPhe-OMe (260)	595	720
2-Adoc-R-α-Me-Trp-(2S,3S)-∇^ZPhe-OH (266)	126	84
2-Adoc-R-α-Me-Trp-(2S,3R)-∇^EPhe-OMe (262)	6.5	-

[a] Binding affinities defined in footnote a, **Table 11**

It can be seen from Table 12 that with respect to CCK-B receptor binding the introduction of a carbon-carbon double bond to the Phe residue with Z-stereochemistry gives reasonable binding of which the acid form (**220**) has a 5-fold increased activity over its ester counterpart (**219**). This latter feature is likely to be due to the presence of H-bonding of the carboxyl group with the enzyme receptor site. Whilst the presence of a cyclopropyl group gives slightly worse binding in both ester and acid forms, (**260**) and (**266**), when present with Z-configuration than the corresponding (Z)-dehydro Phe analogues (**219**) and (**220**) respectively. The ester (**262**), however, with E-configuration exhibits excellent CCK-B binding affinity with an IC_{50} = 6.5nM. The corresponding acid form of (**267**) would thus be expected to have a binding affinity similar to the parent substrate CCK-4 (K_i = 3nM) [367].

The O-acetate Phe derivative (**218**) was also found to have very good CCK-B activity (IC_{50} = 13nM).

Molecular dynamic studies

Molecular dynamic studies were performed upon the three peptides 2-Adoc-α-Me-(R)-∇^{Z}Phe-OMe(**260**), 2-Adoc-α-Me-(R)-∇^{E}Phe-OMe(**262**) and 2-Adoc-α-Me-(R)-Δ^{Z}Phe-OMe(**219Z**) in an attempt to elucidate their preferred conformations. Prior to this, the effect of the α-Me-(R)-Trp residue ;which is common to all three peptides; on the overall peptide conformation was looked at in detail by determining its (ϕ,ψ) energy contour map. The model chosen to study this was the dipeptide N-Ac-α-Me-(R)-Trp-N-Me.

The ϕ,ψ energy map of N-Ac-α-Me(R)-Trp-N-Me indicates two main low energy regions, one on the right hand side of the map at ϕ, 30 to 100°, ψ, -80° to 60° and a similar region on the left hand side of the map at ϕ, -80° to -50°, ψ, -50° to 100° (Fig. 6). The rotational energy barrier between these two conformationally favoured regions being 6-8kcalmol^{-1}. The lowest energy conformation is indicated on the map by an X. It can also be seen that a negative ϕ value prefers a positive ψ value, whilst for a positive ϕ value there is no preference in the sign of the ψ value.

The ten lowest energy minima calculated for the dehydro- and 2,3-methano-phenylalanine derivatives (**219Z, 260** and **262**) are shown in Tables 13-15. In each case the β-turn type stated represents the initial conformation of the dipeptide before minimisation studies were applied; and not the resultant dipeptide conformation, which is defined by the calculated ϕ, ψ and χ values.

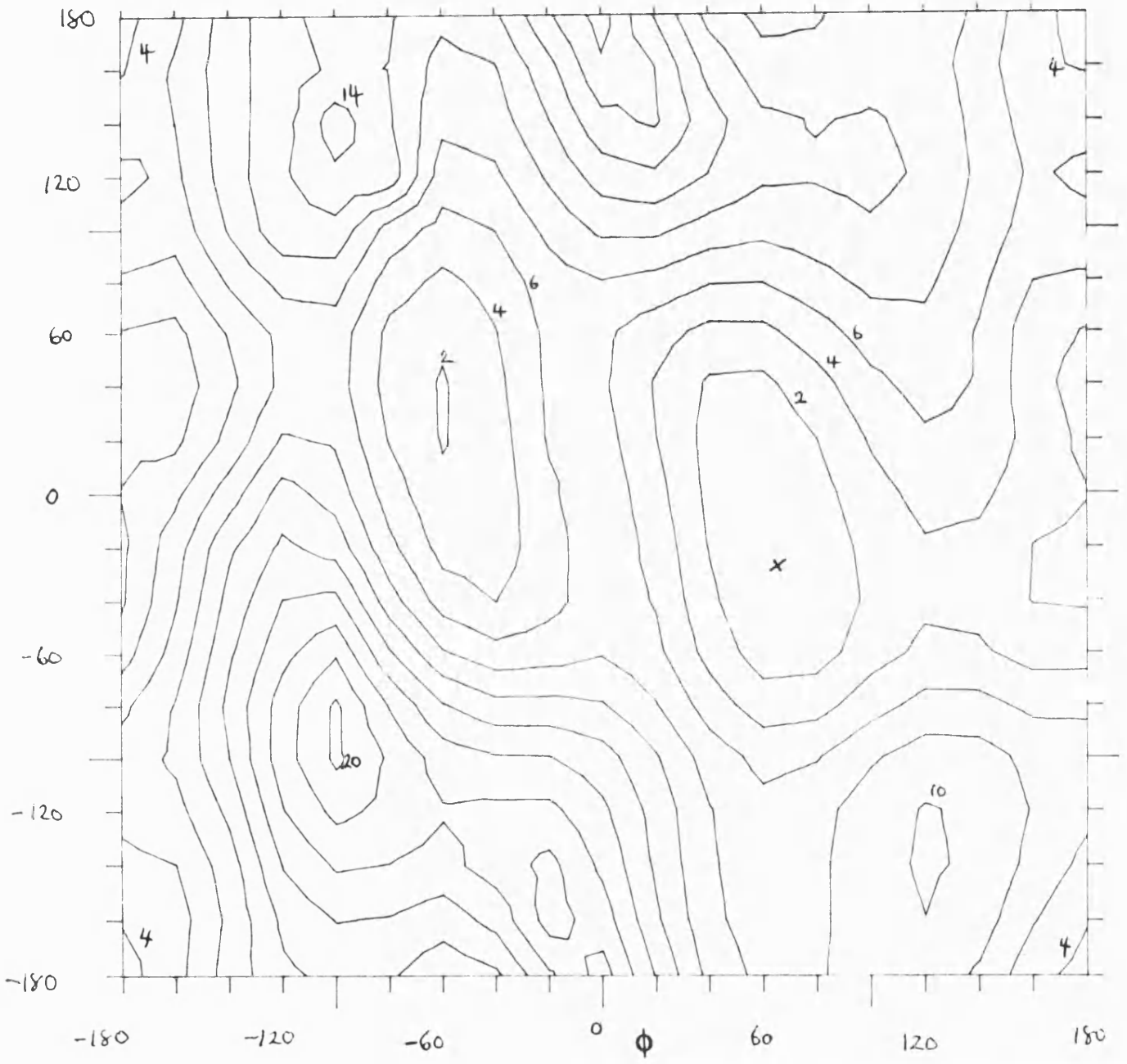


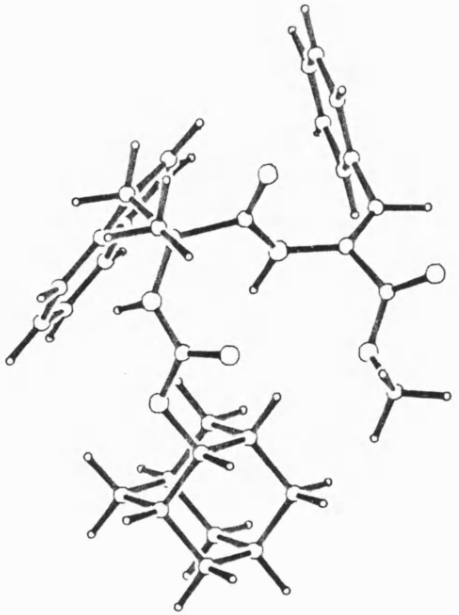
Fig6. A (ϕ,ψ) energy contour map for the dipeptide N-Ac-α-Me-(R)-Trp-N-Me

For 2-Adoc-α-Me-(R)-Trp-Δ^ZPhe-OMe (**219**) the ten calculated minimum energy conformations (Table 13) all possessed a β-turn, but there was no preference in type.

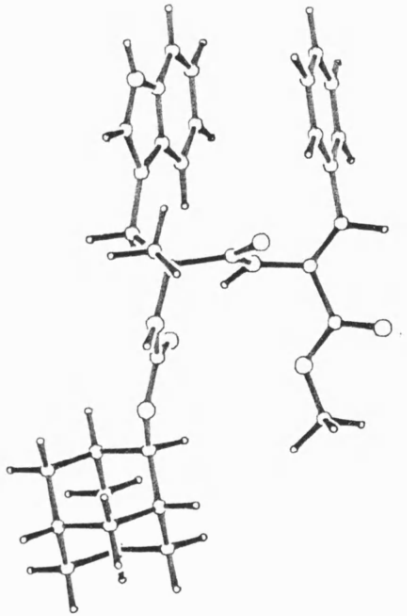
Table 13 Minimum energy conformations of CCK-4 dipeptide analogue 2-Adoc-α-Me-(R)-Trp-Δ^ZPhe-OMe

S.No	β-turn type	α-Me-(R)-Trp			Δ^ZPhe			Rel. Energy Kcal/mol
		ϕ	ψ	χ	ϕ	ψ	χ	
1	I'	45	23	-65	93	43	2	0
2	I'	42	29	-66	94	-72	2	0
3	I	-72	67	53	-81	-26	0	0.6
4	II	-68	73	-178	94	31	0	0.6
5	II	-62	40	-70	96	37	2	1.4
6	I'	53	24	-64	95	40	2	1.5
7	II	-72	32	48	97	30	4	1.8
8	I'	51	24	-70	99	37	2	1.8
9	I	-72	62	54	-93	32	0	1.9
10	I	-72	60	54	-89	-27	-1	1.9

The majority of the conformations favoured the indole and phenyl rings on the same side of the peptide chain, with the adamantyl group on the opposite side (Fig.7).



Structure 1.



Structure 4.

Fig 7. Two minimum energy conformations of 2-Adoc-α-Me-R-Trp-Δ^{Z}-Phe-OMe (Structures 1 and 4)

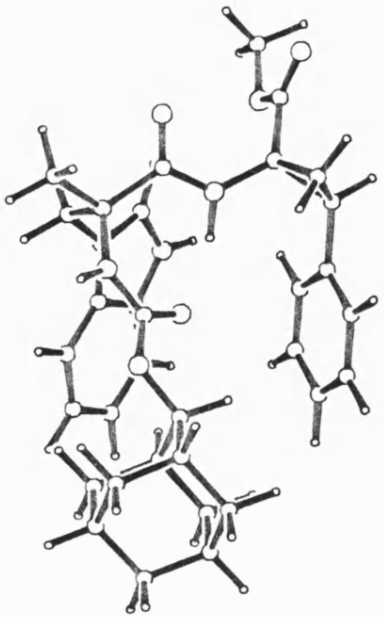
In one particular case, Structure 4, there is clearly π stacking occuring between the phenyl and indole rings, the *inter*-ring distance being 4-6Å.

Similarly for the ∇^{Z}Phe-OMe derivative (**260**) there was no preference in β-turn type [Table 14].

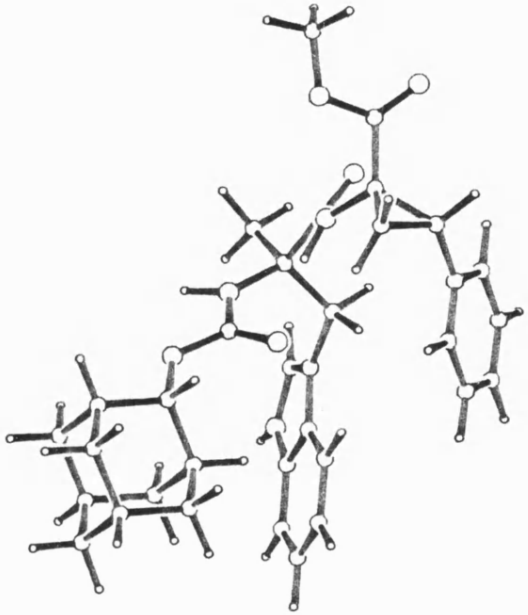
Table 14 Minimum energy conformations of CCK-4 dipeptide analogue 2-Adoc-α-Me-(R)-Trp-∇^{Z}Phe-OMe

S.No	β-turn type	α-Me-(R)-Trp			∇^{Z}Phe			Rel. Energy Kcal/mol
		ϕ	ψ	χ	ϕ	ψ	χ	
1	I	-60	57	-76	-55	-58	8	0
2	II	-67	30	47	71	59	18	1.3
3	I'	40	36	-65	68	59	18	1.8
4	II	-69	25	47	72	59	18	2.5
5	I	-72	56	53	-66	-62	6	2.5
6	I	72	48	160	-84	-72	6	2.7
7	II	-72	55	57	76	60	18	3.1
8	II	-71	58	59	77	59	18	3.6
9	I	-56	4	-73	-67	-65	4	4.1
10	II'	54	20	59	-69	-62	4	4.3

The four lowest energy conformations (Structures 1-4) (Fig.8) have the phenyl and indole rings on the same side of the peptide chain, whilst the remainder had phenyl and indole rings on opposite sides. In all these examples the adamantyl group tends to be in close proximity to either phenyl or indole ring



Structure 1.



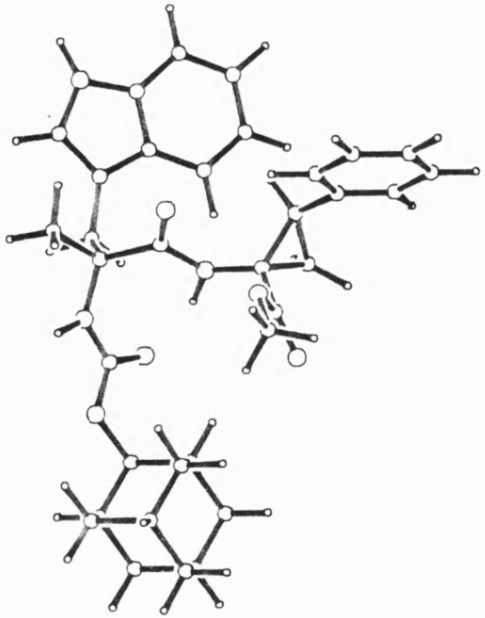
Structure 2.

Fig 8. Two minimum energy conformations of 2-Adoc-α-Me-R-Trp-∇^{Z}Phe-OMe (Structures 1 and 2)

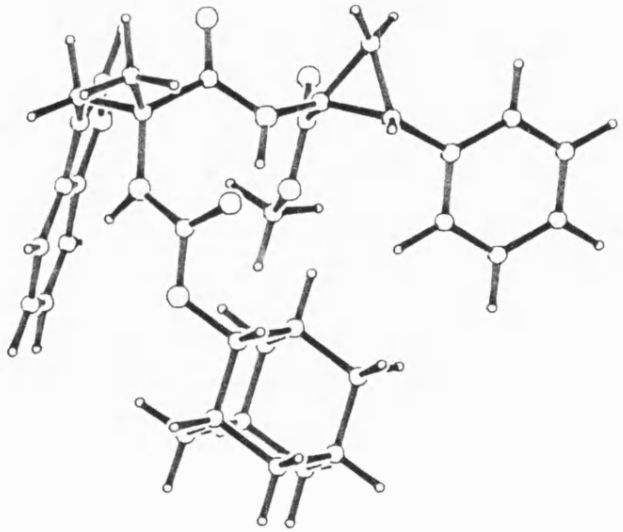
In the case of the ∇^{E}Phe-OMe derivative (**262**) the two lowest energy conformations (Structures 1 and 2) (Fig.9) have the phenyl and indole rings approximately orthogonal to one another. It is interesting to note that energies of structures (1) and (2) are similar even though the relative disposition of the rings are quite different in the two cases. For the remainder of the examples (Structures 3-10) all possess the indole and phenyl groups on the same side of the peptide chain. Once again all β-turn types are favoured.(Table 15).

Table 15 Minimum energy conformations of CCK-4 dipeptide analogue 2-Adoc-α-Me-(R)-Trp-∇^{E}Phe-OMe

S.No	β-turn type	α-Me-(R)-Trp			∇^{E}Phe			Rel. Energy Kcal/mol
		ϕ	ψ	χ	ϕ	ψ	χ	
1	II	-65	61	174	86	-87	150	0
2	II'	54	-13	-71	-75	-57	141	0.3
3	I	-66	71	-177	-54	-56	144	0.4
4	II	-68	59	177	85	-86	150	0.8
5	II	-66	61	176	85	74	151	1.0
6	II	-72	51	53	85	-86	150	2.0
7	II	-68	59	178	84	80	151	2.2
8	I	-70	45	162	-88	-62	141	2.8
9	II'	61	-9	-68	-75	-60	143	2.9
10	I	-54	-6	-75	-76	-63	142	2.9



Structure 1.



Structure 2.

Fig 9. Two minimum energy conformations of 2-Adoc-α-Me-R-Trp-∇^{E}Phe-OMe (Structures 1 and 2)

2.6 Conclusion

The amino acids ∇Phe, ΔPhe and α-Me-(R)-Trp are all highly constrained residues and as a consequence the overall conformation appears to be governed by the constraints imposed by the individual residues present. As a result, the allowed low energy conformations of ∇Phe, ΔPhe and α-Me-(R)-Trp are conducive to all β-turn types. The type of β-turn present may therefore be unimportant but the presence of a β-turn appears to be important.

The majority of low energy conformations, in all three dipeptides, indicated the preference of both indole and phenyl rings to lie on the same side of the peptide chain, and thus be available for receptor binding at the active site. This is in accordance with the CCK-B receptor site model proposed by Horwell *et al* [321].

Future work should be directed towards the synthesis of the remaining diastereomers of ∇Phe and ΔPhe analogues of α-Me-(R)-Trp-Phe especially the E-forms which indicate excellent potential as CCK-B dipeptide mimetics.

CHAPTER 3

EXPERIMENTAL

EXPERIMENTAL

3.1 Instrumentation and experimental techniques

3.1.1 *Solvents and reagents*

All solvents were dried and distilled before use. Petrol refers to petroleum ether boiling in the range 60-80°C. Tetrahydrofuran was pre-dried over sodium wire and then refluxed over sodium benzophenone ketyl under a nitrogen atmosphere until anhydrous. This was redistilled immediately prior to use. All other solvents and reagents were purified using the procedures described in *Purification of laboratory chemicals* [371].

3.1.2 *Chromatography*

Thin layer chromatography (tlc) was used extensively as a qualitative guide during reactions and for assessing the purity of compounds. Merck DC-alufolien Kieselgel 60 F_{254} sheets and Whatman Reverse Phase $KC_{18}F$ octadecylsilane bonded plates (both containing fluorescent indicator) were used for this purpose. Visualisation of compounds was achieved by illumination under short wavelength (254nm) ultraviolet light (when possible). Plates were developed by treatment with either a 0.5% (w/v) aqueous solution of potassium permanganate or a 7% (w/v) methanolic solution of phosphomolydbic acid or a 0.3% (w/v) solution of ninhydrin in butanol or a 3% (v/v) solution of anisaldehyde in ethanol, normally followed by warming of the tlc plate.

Normal phase medium pressure flash chromatography was routinely employed using Amicon Matrex or Merck 9385 silica gel whilst LiChroprep RP-18 reverse phase silica gel was used for reverse phase chromatograhy. Columns were packed as a slurry in the eluting solvent and the material to be chromatographed introduced directly as a solution

in the eluting solvent or preabsorbed onto silica and then applied as a thin layer to the top of the column. A pressure gradient was developed using a small hand bellow.

3.1.3 *General*

Glassware used for moisture sensitve reactions was heated in an oven at 120°C overnight and then allowed to cool in a desicator over calcium chloride. Flasks and stirrer bars were additionally flame dried under a stream of dry nitrogen prior to use.

Solvents were evaporated with a Buchi rotary evaporator using a water aspirator or a vacuum pump as required and a water bath temperature <40°C to avoid unnecessary heating.

3.1.4 *Analysis and spectroscopy*

Melting points (m.p.) were determined on commercially available apparatus (electrothermal MK II or Gallenkamp) and are uncorrected. Elemental micro-analyses were carried out using a Carlo Erba 1106 Elemental Analyser. Optical rotations were measured using a Perkin-Elmer 141 polarimeter with concentrations expressed in $g/100cm^3$.

Infrared spectra were recorded in the range 4000-600cm^{-1} using a Perkin-Elmer 1310 spectrophotometer and peaks are reported (ν_{max}) in wave numbers (cm^{-1}) and the abbreviations br (broad), s (strong) and vs (very strong) were used to describe the peaks. Samples were prepared as liquid films, nujol mulls or chloroform solutions as indicated.

Proton magnetic resonance spectra were recorded on a Jeol GX FT 270 (270MHz) spectrometer, although where indicated a Jeol GX FT 400 (400MHz) instrument was

used. Carbon-13 magnetic resonance spectra were recorded on a Jeol GX FT 270 spectrometer operating at 67.8MHz and using 90 and 135 DEPT pulse sequences to aid multiplicity determination. Chemical shifts (δ) are expressed in parts per million downfield from internal tetramethylsilane. The multiplicities of the resonances are denoted by s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet) and m (multiplet). The abbreviation br (broadened) is used to indicate significant broadening, whether due to rapid exchange or unresolved fine coupling. Homonuclear decoupling experiments and 2D homonuclear shift correlated (COSY) spectra were used to confirm proton assignments when required.

Mass spectra were recorded using a VG Analytical 7070E instrument with a VG 2000 data system. Electron ionisation (E.I.) spectra were produced using an ionising potential of 70eV. Chemical ionisation (C.I.) was employed using *iso*-butane as the reagent gas.

3.2 Experimental procedure

Preparation of dehydropeptides

Preparation of (RS)-threo-3-phenylserine methyl ester (**214**).

To methanol ($100cm^3$) in $250cm^3$ rbf was added dropwise neat thionyl chloride (23ml, 275mmol) with stirring at 0°C. To this was added RS-*threo*-3-phenylserine hydrate (10g, 55mmol) in solid form. After allowing to warm to room temperature the suspension was stirred overnight. The clear solution was neutralised with 1M sodium hydroxide solution, extracted with DCM ($3x75cm^3$) and the combined organic extracts dried (sodium sulphate), filtered and the solvent was removed *in vacuo* to yield a yellow oil (**214**), (8.6g, 80%) (R_f=0.58, methanol-DCM (15:85)); υ_{max} ($CHCl_3$) 3373

and 3200 ($NH_2$and OH), 1739 (CO_2Me), 1670 (Ar C=C), 1216 (C-N); δ_H ($CDCl_3$) 2.44 (3H, s, NH_2 and OH), 3.62 (1H, d, $J_{3,2}$4.6, 3-H); 3.67 (3H, s, CO_2Me), 4.90 (1H, d, $J_{2,3}$4.6, 2-H), 7.33 (5H, s, Ph).

N^{α}-(2-Adamantyloxycarbonyl)-α-methyl-R-tryptophanyl-R,S-threo-3-phenylserine methyl ester (**215**)

To a solution of the acid (**206**) (1g, 2.5mmol) in ethyl acetate (50cm^3) was added 1-hydroxybenzotriazole (0.42g, 2.8mmol) in solid form. This was followed by the dropwise addition of a solution of N,N′-dicyclohexylcarbodiimide (0.62g, 3.0mmol) in ethyl acetate (5cm^3) at room temperature. The suspension was stirred for one hour. The precipitate which had formed (dicyclohexylurea) was filtered off and a solution of 3-phenylserine methyl ester (**214**) in ethyl acetate (10cm^3) was added dropwise to the filtrate. After 23h the solution was worked up by washing with 5% aqueous citric acid solution (25cm^3), saturated aqueous sodium bicarbonate solution (25cm^3) and brine (30cm^3). The organic phase was dried over anhydrous magnesium sulphate and reduced *in vacuo* to yield a yellow oil. After purification *via* flash chromatography on silica gel by elution with methanol-DCM (1:19), the desired product (**215**) was isolated as a white foam (1.2g, 83%); (R_f=0.69, methanol-DCM (15:85), υ_{max} ($CHCl_3$) 3395 br (OH and NH_2), 1734 (CO_2Me), 1670 (CONH), 1496 (Ar C=C), 1215 (C-N); δ_H ($CDCl_3$), 1.45 (3H, s, R-α-Me), 1.46-1.96 (14H, m, adamantyl), 3.23 (1H, d, J_{gem} 15, 3-H.1), 3.41 (1H, d, J_{gem} 15, 3-H.1), 3.61 (3H, s, CO_2Me), 4.82 (1H, br s, adamantyl 2-H), 4.92 (1H, m, 2-H.2), 5.09 (1H, d, *J* 4.4, 3-H.2), 5.28 (1H, br s, 2-NH.2), 5.32 (1H, s, 2-NH.1), 6.80-7.57 (10H, m, Indole, Ph Ar), 8.34 (1H, s, Indole NH); δ_C ($CDCl_3$) 14.14 (R-α-Me), 21.02 (Ad), 23.71 (Ad), 24.55 (Ad), 25.23 (Ad), 26.86 (Ad), 27.05 (Ad), 31.66 (C-3.1), 31.98 (Ad), 33.12 (Ad), 36.26 (Ad), 37.27 (Ad), 52.35 (C-2.2), 58.64 (MeO), 60.45 (C-2.1), 74.30 (C-3.2), 109.57 (Ar), 111.09 (Ar), 118.88 (Ar), 119.52 (Ar), 121.83 (Ar), 123.87 (Ar), 124.03 (Ar), 125.91 (Ar), 127.89 (Ar), 128.33

(Ar), 135.87 (Ar), 139.53(AR), 155.4(CONH), 170.80(OCONH), 174.31(CO_2Me); m/z (+FAB), 574(MH^+,27%), 444(17), 378(20),135(100).

*Attempted dehydration of the alcohol (**215**) to yield the dehydroamino acid, N^{α}-(2-adamantyloxycarbonyl)-α-methyl-R-tryptophanyl-dehydro phenylalanine methyl ester* (**219Z**)

Method 1.

To a solution of the alcohol (**215**) (100mg, 0.17mmol) in dry THF (5cm^3) was added pyridine (14µl, 0.17mmol) followed by diethylaminosulphur trifluoride (23µl, 0.17mmol) with stirring under nitrogen at 0°C. After stirring overnight the solution had turned a light brown colour, however no presence of the dehydrated product was observed except baseline material by tlc, solvent methanol-DCM (1:9). After a further 24 hours the solution was worked up by washing with water (5cm^3) and extracting with DCM (3x10cm^3). The combined organic extracts were dried over sodium sulphate and reduced *in vacuo* yielding a dark brown oil. Purification *via* flash chromatography on silica gel gave only recovered starting material (**215**) (20mg, 20%).

Method 2.

To a solution of the alcohol (**215**) (100mg, 0.17mmol) in THF (3cm^3) was added triethylamine (24µl, 0.17mmol) followed by N,N′-carbonyldiimidazole (28mg, 0.17mmol) with stirring under nitrogen at room temperature. After 3h no product had been formed. The solution was refluxed for 24h and worked up by washing with water (5cm^3) and extracting into DCM (3x10cm^3). The combined extracts were dried over anhydrous sodium sulphate and reduced *in vacuo* to give a pale yellow oil. ^1H NMR spectroscopy revealed only the presence of recovered starting material (Yield 80%).

Method 3.

To the alcohol (**215**) (100mg, 0.17mmol) and dried sodium acetate (anhydrous) (28mg, 0.34mmol) was added acetic anhydride ($3cm^3$) with stirring at 30°C. The solution immediately turned from colourless through orange to red. After 12h the reaction was complete. The solution was washed with water ($5cm^3$) and extracted with DCM ($3x30cm^3$). The combined organic extracts were dried over anhydrous magnesium sulphate and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with methanol-DCM (2:98). No dehydroamino acid was generated but instead (**218**) (O-acetylated product) was isolated as a pale yellow foam (104mg, 100%), (R_f=0.61 (methanol-DCM (1:9)); (Found C, 66.14; N, 6.61, H, 6.77 $C_{35}H_{41}N_3O_7$. H_2O required C, 66.2; N, 6.23; N, 6.92). υ_{max} ($CHCl_3$) 3395 (amide NH), 1747 vs br (CO_2Me and CH_3CO), 1496 (Ar C=C), 1232 (C-O and C-N); δ_H ($CDCl_3$) 1.38-2.10 (17H, m, adamantyl and R-α-Me), 2.10 (3H, d, acetyl), 3.20 (1H, d, J_{gem} 15, 3-H.2), 3.51 (1H, d, J_{gem} 15, 3-H.2), 3.61 (3H, d, CO_2Me), 4.84 (1H, s, adamantyl 2-H), 5.02 (1H, m, 2-H.2), 5.08 (1H, s, 2-NH.2), 5.18 (1H, s, 2-NH.1), 6.24 (1H, m, 3-H.2), 6.80-7.57 (10H, m, Ph, indole Ar), 8.75 (1H, d, indole NH); δ_C ($CDCl_3$) 13.82 (R-α-Me), 20.40 (Ac Me), 23.22 (Ad), 23.58 (Ad), 24.59 (Ad), 25.20 (Ad), 26.60 (Ad), 26.79 (Ad),31.36 (C-3.1), 31.69 (Ad), 33.34 (Ad), 36.00 (Ad), 36.98 (Ad), 48.78 (Ad),52.22 (C-2.2), 56.55 (MeO), 60.30 (C-2.1), 74.26 (C-3.2), 108.79 (Ar), 111.09(Ar), 118.39 (Ar), 119.04 (Ar), 121.34 (Ar), 124.00 (Ar), 126.08 (Ar),128.12 (Ar), 135.61 (Ar), 135.84 (Ar), 155.20 (CONH), 169.44 (OC=O),174.47 (CO_2Me), 174.63 (Ac C=O); *m/z* (-FAB) 635 (M^+, missing), 614 ($M-H_3O^+$, 90%), 402 (100), 314 (40), 273 (38).

Method 4.

To a mixture of the alcohol (**215**) (100mg, 0.17mmol) and N,N′-disuccinimidyl carbonate (44mg, 0.17mmol) under nitrogen was added dry acetonitrile ($3cm^3$)

followed by triethylamine (24μl, 0.17mmol) with stirring at room temperature. After 2d the suspension was worked up by washing with water ($5cm^3$) and extracting into DCM ($3x10cm^3$). The combined organic extracts were dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash chromatography eluting with methanol-DCM (2:98) to give the desired dehydroamino acid (**219Z**) (46mg, 49%) as a white foam, m.p. 177-182°C (R_f=0.64) Methanol-DCM (1:9), (Found C, 70.76; H, 6.83; N, 7.29. $C_{33}H_{37}N_3O_5$. 0.25 H_2O requires C, 70.84; H, 6.71; N, 7.51), $[\alpha]^{22}_D$ +26° (c 3.6 in $CHCl_3$). υ_{max} ($CHCl_3$) 3366 (amide NH), 1713 vs br (CO_2Me, CONH, and conj. C=C) 1490 (Ar, C=C), 1214 (C-N); δ_H ($CDCl_3$) 1.48-1.95 (17H, m, adamantyl and R-α-Me), 3.33 (1H, d, J_{gem} 15, 3-H.1), 3.68 (1H, d, J_{gem} 15, 3-H.1), 3.83 (3H, s, CO_2Me), 4.86 (1H, s, adamantyl 2-H), 5.14 (1H, s, 2-NH.1), 7.05-7.64 (11H, m, Ph, indole Ar, and 3-H.2 (7.33)), 8.20 (1H, s, 2-NH.2), 8.24 (1H, s, indole NH); δ_C ($CDCl_3$)24.00 (Ad), 26.89 (Ad), 27.08 (Ad), 31.33 (Ad), 31.69 (C-3.1), 32.01 (Ad), 32.08 (Ad), 36.33 (Ad), 37.30 (Ad), 52.58 (MeO), 61.11 (C-2.1), 109.86 (Ar), 111.12 (Ar), 119.04 (Ar), 119.82 (Ar), 122.12 (Ar), 124.13 (Ar), 124.36 (Ar), 128.41 (Ar), 129.16 (Ar), 129.81 (Ar), 131.13 (Ar), 133.76 (Ar), 155.00 (CONH), 165.00 (OC=O), 173.00 (CO_2Me); *m/z* (C.I., *iso*-butane) 636 (MH^+ missing) (MH^+ - 0.25 H_2O, 18%), 524 (8), 468 (7), 404 (79), 135 (100).

Attempted hydrolysis of the ester (**219Z**)

Method 1.

To a solution of the ester (**219Z**) (100mg, 0.18mmol) in THF ($3cm^3$) was added a 0.1M lithium hydroxide solution ($2cm^3$, 0.2mmol) with stirring at room temperature. The suspension was then refluxed overnight, however tlc indicated that no hydrolysis had occurred. The solution was worked up and starting material (**219Z**) (95mg, 95%) was recovered.

Method 2.

To a solution of the ester (**219Z**) (78mg, 0.14mmol) in ethanol ($10cm^3$) was added a 0.1M sodium hydroxide solution ($1.5cm^3$, 0.15mmol) with stirring at room temperature. After 5h reflux, complete hydrolysis had been achieved (as determined by tlc). The solution was then neutralised with 0.1M hydrochloric acid, washed with water ($5cm^3$) and extracted into ethyl acetate ($3x5cm^3$). The combined organic extracts were dried over anhydrous magnesium sulphate and concentrated *in vacuo*. The residue was purified by reverse-phase chromatography using methanol-water (3:1) as eluant to give the acid (**220Z**) (30mg, 40%) as a white foam ; (R_f=0.14, methanol-DCM (1:9)), $[\alpha]^{22}_D$ +34° (c 3.6 in $CHCl_3$); υ_{max} (Nujol) 3265 (OH), 1715 s (CO_2H, CONH, C=C), 1240 (C-N); δ_H ($CDCl_3$), 1.38-1.87 (17H, m, adamantyl, R-α-Me), 3.24 (1H, d, J_{gem} 14.8, 3-H.1), 3.53 (1H, d, J_{gem} 14.8, 3-H.1), 4.77 (1H, s, adamantyl 2-H), 5.06 (2H, br s, CO_2H, 2-NH.1), 6.98-7.52 (11H, m, Ph, indole and 3-H.2), 8.15 (1H, s, 2-NH.2), 8.52 (1H, s, indole NH); δ_C ($CDCl_3$) 14.04 (R-α-Me), 22.96 (Ad), 23.68 (Ad), 24.78 (Ad), 26.86 (Ad), 27.05 (Ad), 28.90 (Ad), 29.68 (Ad), 30.33 (Ad), 30.97 (Ad), 31.66 (C-3.1), 31.98 (Ad), 36.26 (Ad), 37.27 (Ad), 37.53 (Ad), 61.20 (C-2.1), 88.15 (C-2.2), 109.11 (Ar), 111.35 (Ar), 118.78 (Ar), 119.69 (Ar), 121.96 (Ar), 123.93 (Ar), 124.49 (Ar), 128.35 (Ar), 128.48 (Ar), 129.55 (Ar), 129.93 (Ar), 130.88 (Ar), 133.47 (Ar), 136.03 (Ar), 155.00 (CONH), 168.73 (OC=O), 173.24 (CO_2H); *m/z* (+FAB) 542 (MH^+, 10%), 412 (8), 391 (20).

Attempted cyclopropanation of the dehydropeptide (**219Z**)

To a solution of the dehydroamino ester (**219Z**) (50mg, 0.09mmol) in ether ($5cm^3$) was added an ethereal solution of diazomethane ($5cm^3$, 0.09mmol, 1mol eq)(prepared by method 2, described on page 156) with stirring at room temperature. After 1d, some starting material was still present (as determined by tlc) and so an excess of ethereal

diazomethane (0.45mmol, 5mol eq) was added. The solution was stirred for one day and worked up by quenching the excess diazomethane with glacial acetic acid and subsequent concentration *in vacuo*. ^{1}H NMR of the resultant residue showed a multi-component mix, of which none corresponded to the desired cyclopropyl dipeptide.

Preparation of 2,3-methanophenylalanine derivatives

(4S)-iso-Propyl-1,3-oxazolidin-2,5-dione (**221**)[372]

To a suspension of L-valine (5.85g, 0.05mol) in dry THF (75cm^3) was added a 20% phosgene/toluene solution (54.9cm^3, 0.1mol) whilst under an atmosphere of nitrogen. The solution was stirred at 40°C until clear, the solution was purged with dry nitrogen for 2h and the solvent removed *in vacuo*. The residue was taken up in a little dry THF and the solvent once again removed *in vacuo* (removal of hydrogen chloride). A white, pungent solid (**221**)(7.21g, 99.3%) resulted after drying under vacuum for 3h; υ_{max} (liquid film) 3290 s (NH), 1751 (C=O), 1620-1670 (C=O); δ_H ($CDCl_3$) 1.03 (3H, d, *J* 7, *i*-Pr Me), 1.09 (3H, d, *J* 7, *i*-Pr Me), 2.26 (1H, m, *i*-Pr CH), 4.25 (1H, d, $J_{4,6}$ 4.2,4-H), 7.25 (1H, s, NH).

(6S)-iso-Propylpiperazin-2,5-dione (**222**).

A solution of (**221**) (18.4g, 0.13mol) in dry THF (130cm^3) was added dropwise to a stirring mixture of glycine ethyl ester hydrochloride (18.0g, 0.13mol), triethylamine (38cm^3, 0.39mol) and dry chloroform (210cm^3) (in a 500cm^3 2-necked rbf) at -78°C. After 3h stirring, the mixture was allowed to warm to room temperature, the triethylamine hydrochloride salt was filtered off under vacuum and the solvent was evaporated *in vacuo*. Toluene (210cm^3) was added to the residue, the resulting suspension was heated for 12h under reflux with mechanical stirring, and then cooled

to 0°C. (**222**) was removed by suction filtration, washed several times with ether and then dried *in vacuo* at 100°C. (**222**) (12.2g, 60%) was a white solid; m.p. 253°C (from water) (lit.[372], 254°C); υ_{max} (nujol) 3360 (amide NH), 1670 (CONH), 1215 (C-N); δ_H (d^6-DMSO) 0.86 (3H, d, *J* 7, *i*-Pr Me), 0.93 (3H, d, *J* 7, *i*-Pr Me), 2.11 (1H, m, *i*-Pr CH), 3.54 (1H, d, $J_{6,7}$ 3.7, 6-H), 3.63 (1H, d, $J_{3,3}$ 18, 3α-H), 3.83 (1H, d, $J_{3,3}$ 18, 3β-H), 8.03 (1H, s, NH), 8.21 (1H, s, NH).

Acylation of the piperazin-2,5-dione (**222**)

Preparation of (6S)-1,4-diacetyl-6-iso-propylpiperazin-2,5-dione (**226**)

Method 1.

To the piperazin-2,5-dione (**222**) (1.56g, 10mmol), previously dried *in vacuo* at 120°C for one day, was added acetic anhydride (60cm^3). The suspension was heated, with stirring, to 110°C for 7h. The solvent was removed *in vacuo* and the product (**226**) was isolated *via* column chromatography on silica gel by elution with ethyl acetate-petrol (1.5:8.5). The desired product (**226**) was isolated as a pale yellow solid (1.86g, 78%); m.p. 73-4°C (ethyl acetate-petrol) (R_f=0.36 ethyl acetate-petrol (2:8)). (Found C, 55.3; H, 6.87; N, 11.7. $C_{11}H_{16}N_2O_4$ requires C, 55.00; H, 6.67; N, 11.67); υ_{max} (liquid film) 2870-2960, 1690 (C=O), 1370; δ_H ($CDCl_3$) 0.99 (3H, d, *J* 6.8, *i*-Pr Me), 1.11 (3H, d, *J* 6.8, *i*-Pr Me), 2.05 (1H, m, *i*-Pr CH), 2.57 (3H, s, N-Ac), 2.60 (3H, s, N-Ac), 4.11 (1H, d, $J_{3,3}$ 19.1, 3α-H), 5.01 (1H, d, $J_{6,7}$ 9.7, 6-H), 5.10 (1H, d, $J_{3,3}$ 19.1, 3β-H); δ_C ($CDCl_3$) 19.27 (2 x *i*-Pr Me), 26.53 (Ac Me), 26.99 (Ac Me), 31.43 (C-7), 46.84 (C-3), 62.44 (C-6), 166.52 (CONH), 167.14 (CONH), 170.84 (Ac C=O), 171.29 (Ac C=O); *m/z* (E.I.) 240 (M^+, 40%), 198 (100, M-C_2H_2O), 156 (90, 198-C_2H_2O), 114 (20).

Method 2.

To a suspension of the piperazin-2,5-dione (**222**) (1g, 6.4mmol) in DMF (15cm^3) was added acetic anhydride (1.3cm^3, 14.1mmol, 2.2mol eq) with stirring. The suspension was refluxed for 4h, the solvent was removed *in vacuo* and the brown residue was purified by flash chromatography on silica in the usual manner (method 1) to yield **226** as a pale yellow solid (0.7g, 45%). (Previously characterised).

Method 3.

To a suspension of the piperazin-2,5-dione (**222**) (20.1g, 0.13mol) in acetic anhydride (200cm^3) was added pyridine (23.1cm^3, 0.29mol, 2.2mol eq) with stirring. The suspension was refluxed for 7h, the solvent was removed *in vacuo* to yield a brown residue. This was purified in the usual manner to yield **226** (17.8g, 57%). (Previously characterised).

Method 4.

To a suspension of the piperazin-2,5-dione (**222**) (1g, 6.4mmol) in acetic anhydride (10cm^3) was added pyridine (1cm^3, 12.8mmol, 2mol eq) followed by ultrasonification for 3d. The solvent was then removed *in vacuo* and the residue purified by flash chromatography in the usual manner. The diacylpiperazin-2,5-dione (**226**) was isolated in low yield (0.44g, 29%). (Characterised previously).

Method 5.

To a suspension of the piperazin-2,5-dione (**222**) (1g, 6.4mmol) in dry DMF (15cm^3) was added acetyl chloride (1cm^3, 14.2mmol, 2.2mol eq) with stirring. The suspension was refluxed for 6h, the solvent was reduced *in vacuo* and the residue purified by flash chromatography in the usual manner. To yield **226** (0.66g, 43%) as a yellow crystalline

solid (characterised previously).

Method 6.

To a suspension of the piperazin-2,5-dione (**222**) (1g, 6.4mmol) in acetyl chloride ($10cm^3$) was added DBU (2.1g, 14.1mmol, 2.2mol eq) with stirring at 0°C. The suspension instantly turned pale green on the additon of DBU, the intensity of which increased as the reaction progressed. After 1h the solvent was removed *in vacuo* and the pale brown residue was purified by flash chromatography in the usual manner to yield **226** (0.48g, 33%) as a yellow solid (previously characterised).

Preparation of

(6S)-N(1)-acetyl-3-benzylidene-6-iso-propylpiperazin- 2,5-dione (**227**)

A solution of potassium *t*-butoxide (1.3g, 11.5mmol, 1.1mol eq) in dry THF ($20cm^3$) was added dropwise to a stirred solution of **226** (2.40g, 10mmol), benzaldehyde (1.06g, 10mmol) and dry DMF ($125cm^3$) at 0°C. After one hour the solution was allowed to warm to room temperature and stirred for a further 14h at which point the solution was quenched with water ($300cm^3$) and extracted with DCM ($3x100cm^3$). The solvent was removed *in vacuo* and the residue was purified *via* column chromatography on silica gel with ethyl acetate-petrol (1:9) as eluent. The major component **227Z** (Z-isomer) (2.35g, 82%) was obtained as a colourless oil (R_f=0.24, ethyl acetate-petrol (2:8)): (Found C, 67.2; H, 6.72; N, 9.69. $C_{16}H_{18}N_2O_3$ requires C, 67.13; H, 6.29; N, 9.79); υ_{max} (liquid film) 3365 (NH), 1670 (C=O), 1628 (C=C), 1445 ; δ_H ($CDCl_3$) 1.05 (3H, d, *J* 1.3, *i*-Pr Me), 1.07 (3H, d, *J* 1.3, *i*-Pr Me), 2.14 (1H, m, *i*-Pr CH), 2.59 (3H, s, N-Ac), 4.98 (1H, d, $J_{6,7}$ 7, 6-H), 7.15 (1H, s, vinyl H), 7.44 (5H, m, Ph), 8.00 (1H, s, NH); δ_C ($CDCl_3$) 18.42 (*i*-Pr Me), 19.17 (*i*-Pr Me), 26.40 (Ac Me), 33.76 (C-7), 61.17 (C-6), 119.69 (C-9), 126.33 (C-3), 128.80 (Ph), 129.32 (Ph), 129.42 (Ph), 132.66 (Ph), 161.69 (CONH), 165.48 (CONH), 171.65 (Ac C=O); *m/z* (E.I.) 286 (M^+, 70%), 244 (15,

M-C_2H_2O), 202 (100, 244 - C_3H_6), 201 (45), 45 (100).

A second product was isolated in a very low yield (30mg, 1%) as a colourless oil (R_f=0.12, ethyl acetate-petrol (2:8)), this was assigned to the E-isomer (**227E**); υ_{max} (liquid film) 3465 (NH), 1670 vs (C=O), 1620 s (C=C); δ_H ($CDCl_3$) 1.08 (3H, d, *J* 6.8, *i*-Pr Me), 1.11 (3H, d, *J* 6.8, *i*-Pr Me), 2.19 (1H, m *i*-Pr CH), 2.52 (3H, s, N-Ac), 4.98 (1H, d, $J_{6,7}$ 7.5, 6-H), 6.65 (1H, s, vinyl H), 7.49 (5H, m, Ph), 9.65 (1H, s, NH); δ_C ($CDCl_3$) 18.49 (*i*-Pr Me), 18.97 (*i*-Pr Me), 26.73 (Ac Me), 33.38 (C-7), 60.88 (C-6), 125.91 (C-3), 126.98 (C-9), 128.12 (Ph), 128.93 (Ph), 129.81 (Ph), 133.08 (Ph), 160.91 (CONH), 168.21 (CONH), 171.84 (Ac C=O); *m/z* (E.I.) 286 (M^+, 100%), 244 (15, M-C_2H_2O), 202 (70, 244-C_3H_6), 201 (25).

Photoequlibration of the (Z)-benzylidene (**227Z**) *into its (E)-geometrical isomer* (**227E**).

Into a quartz test tube was placed a solution of the (Z)-benzylidene (**227Z**) (3g, 10.5mmol) in freshly distilled chloroform (30cm^3). The sample was irradiated with L-W U.V. light (λ=370nm) with stirring for 2 days. The solution was then reduced *in vacuo* to yield a 2:1 mix of Z/E isomers. The two components were separated *via* flash chromatography on silica eluting with ethyl acetate-petrol (1:9).

Attempted cyclopropane construction via the Simmons-Smith reaction

Method 1. Using Zn/Cu couple.

To a solution of the (Z)- benzylidene (**227Z**) (1.28g, 4.5mmol) in ether (30cm^3) containing Zn/Cu powder (0.45g, 6.3mmol of Zn), was added methylene diiodide (3.7cm^3, 45mmol, 10mol eq) and one small crystal of iodine. The reaction mixture was gently heated to reflux for 6h yielding no apparent change by tlc; therefore it was

transferred to an ultrasound bath for a further 3h but still no change was observed, as determined by tlc. The solvent was removed *in vacuo* and the residue was taken up in DCM ($30cm^3$), the couple filtered off and the filtrate was dried over anhydrous Na_2SO_4. The filtrate was reduced *in vacuo* and starting material (1.21g, 95%) was recovered.

Method 2. Using Zn/Ag couple.

To a Zn/Ag couple (0.34g, 4.9mmol of Zn) in dry ether ($10cm^3$) was added a solution of the (Z)-benzylidene (**227Z**) (1g, 3.5mmol) in dry ether ($20cm^3$) with overhead mechanical stirring. To this was added dropwise methylene diiodide (1.3g, 4.9mmol, 1.4mol eq). The mixture was stirred overnight at room temperature and then brought to reflux. After 12h the Zn/Ag couple was filtred whilst hot and the filtrate cooled to 0°C, to which was added ether ($20cm^3$) and pyridine ($3cm^3$). The white precipitate generated (pyridine salts) was filtered through celite and more pyridine was added to the filtrate with refiltration, until all of the iodide salts were removed. The remaining filtrate was reduced *in vacuo* and the residue was purified by flash chromatography on silica eluting with ethyl acetate-petrol (2:8). No desired cyclopropane was isolated. Unreacted starting material (0.6g, 60%) was retrieved.

Preparation of diazomethane

Method 1.

Using the Aldrich mini diazald kit an ethereal diazomethane solution was prepared as follows:

Into a $250cm^3$ rbf, fitted with distillation head, water condenser, receiver adaptor and collecting rbf, was placed potassium hydroxide (6.2g, 0.11mol), water ($10.5cm^3$), ether

(10.5cm^3) and ethanol (37cm^3). The distilling flask was heated in a water bath at 70-75°C with stirring ("Teflon" coated stirrer bar) and a solution of N-nitroso-N'-methyl-*p*-methyltoluene sulphonamide in ether (200cm^3) was added at a rate approximately equivalent to the rate of condensing distillate. Once all the nitrosamide solution had been added, additional ether was placed in the dropping funnel and added at the same rate until the distillate was colourless. The distillate contained 2.8-3.0g (64-69%) of diazomethane. For safety purposes see De Boer and Backer [352].

Method 2.

To a solution of N-methyl-N′-nitroso urea (2.5g, 24mmol)[373] in ether (25cm^3) in a non-quickfit conical flask was slowly added a 40% potassium hydroxide solution (7.5cm^3) with cooling to 0/-5°C (ice-salt bath). On addition of all the potassium hydroxide solution the sample was allowed to warm until all the urea dissolved giving a clear yellow solution. If any urea remained, further potassium hydroxide solution was added until dissolution resulted. The ethereal layer was then decanted into a second flask containing potassium hydroxide pellets (≈12). This was repeated into a third flask. The resultant solution contained approximately 0.7g (17mmol) of diazomethane.

Direct addition in the light

Attempted cyclopropane construction via the addition of diazomethane to the benzylidene (**227**)

To a solution of the (Z)-benzylidene (**227Z**) (2.0g, 7mmol) in dry ether (60cm^3) was added a freshly distilled ethereal solution of diazomethane (8.75mmol, 1.1 mol eq, method 1, page 156) whilst stirring under nitrogen at RT. After 22h the excess diazomethane was quenched *via* the addition of glacial acetic acid (3cm^3). The solvent

was then removed *in vacuo* and the residue was purified by column chromatography on silica gel with ethyl acetate-petrol (0.3:9.7) as eluant. No cyclopropane was generated, but instead (**234**) (O-methylated product) (0.34g, 16%) was isolated as a colourless oil (R_f=0.61, ethyl acetate-petrol (3:97) (Found C, 67.9; H, 6.72; N, 9.25. $C_{17}H_{20}N_2O_3$ requires C, 68.00; H, 6.66; N, 9.33); $[\alpha]^{20}_D$ +258° (c 0.69 in $CHCl_3$); υ_{max} (liquid film) 1680 s (C=O), 1640 (C=N), 1605 (C=C); δ_H ($CDCl_3$) 0.88 (3H, d, *J* 6.9, *i*-Pr Me), 0.98 (3H, d, *J* 6.9, *i*-Pr Me), 2.08 (1H, m, *i*-Pr CH), 2.61 (3H, s, N-Ac), 3.97 (3H, s, MeO), 5.02 (1H, d, $J_{6,9}$ 6.1, 6-H), 7.27 (1H, s, vinyl H), 8.06-7.33 (5H, m, Ph); δ_C ($CDCl_3$) 18.33 (*i*-Pr Me), 19.46 (*i*-Pr Me), 26.73 (Ac Me), 34.11 (C-7), 54.10 (MeO), 58.32 (C-6), 127.99 (Ph), 128.38 (Ph), 129.06 (Ph), 131.78 (C-9), 132.66 (C-3), 135.06 (Ph),163.86 (CONH),164.54 (C-5), 172.30 (Ac C=O); *m/z* (E.I.) 300 (M^+, 100%), 258 (20, M-C_2H_2O), 216 (8), 215 (25, 258-C_3H_7).

A second product was also isolated (**233**) (0.68g, 32%) (R_f=0.43 (ethyl acetate-petrol (2:8)) as a white crystalline solid corresponding to the N-methylated product; m.p. 110-14°C; (Found C, 67.7; H, 6.66; N, 9.33. $C_{17}H_{20}N_2O_3$ requires C, 68.00; H, 6.67; N, 9.33); $[\alpha]^{20}_D$-559° (c 1.16 in EtOH); υ_{max} (liquid film) 1700 vs (C=O), 1666, 1615 (C=C), 1350 vs (C-N); δ_H ($CDCl_3$) 0.98 (3H, d, *J* 6.8, *i*-Pr Me), 1.13 (3H, d, *J* 6.8, *i*-Pr Me), 2.07 (1H, m, *i*-Pr CH), 2.62 (3H, s, N-Ac), 2.91 (3H, s, N-Me), 5.01 (1H, d, $J_{6,7}$ 9.9, 6-H), 7.28-7.43 (6H, m, Ph and vinyl H); δ_C ($CDCl_3$) 19.46 (2 x *i*-Pr Me), 26.56 (Ac Me), 32.53 (C-7), 34.48 (N-Me), 61.66 (C-6), 124.26 (C-9), 128.74 (Ph), 129.38 (Ph), 129.55 (Ph), 132.00(C-3), 133.05 (Ph), 163.96 (CONH), 167.07 (CONH), 171.42 (Ac-C=O); *m/z* (E.I.) 300 (M^+, 100%), 258 (20, M-C_2H_2O) 243 (8, 258-CH_3), 216 (60), 215 (50).

Preparation of (Z)-(1S, 3S, 6S)-N(7)-acetyl-6-iso-propyl-5-methoxy-1-phenyl-4,7-diazaspiro[2,5]oct-4-en-8-one (**237**)

To a solution of the Z-benzylidene (**227Z**) (4.28g, 15mmol) in dry ether ($40cm^3$) was added a freshly distilled ethereal solution of diazomethane (105mmol, 7mol eq, method 1, page 156) with stirring, under nitrogen at RT. After 19h, the excess diazomethane was quenched *via* the addition of glacial acetic acid ($5cm^3$). The solvent was removed *in vacuo* and the residue was purified *via* column chromatography on silica gel using ethyl acetate-petrol (0.5:9.5) as eluant. The desired product (**237**) was isolated as a white solid (1.41g, 30%) m.p.62-3°C, (R_f=0.66 ethyl acetate-petrol (2:8)) (Found C, 68.50; H, 6.91; N, 9.19. $C_{18}H_{22}N_2O_3$ requires C, 68.79; H, 7.01; N, 8.92), $[\alpha]^{20}_D$ -177° (c 1.46 in $CHCl_3$); υ_{max} (liquid film) 1720-1600 vs br (C=N and C=O); δ_H ($CDCl_3$) 0.57 (3H, d, *J* 6.8, *i*-Pr Me), 0.84 (3H, d, *J* 7.0, *i*-Pr Me), 1.88 (1H, m, *i*-Pr CH), 1.89 (1H, dd, J_{22} 4.8, $J_{2B,1}$ 8.4, 2B-H), 2.38 (1H, dd, J_{22} 4.8, $J_{2A,1}$ 9.9, 2A-H), 2.53 (3H, s, N-Ac), 2.88 (1H, dd, $J_{1,2B}$ 8.4, $J_{1,2A}$ 9.9, 1-H), 3.50 (3H, s, MeO), 4.88 (1H, d, *J* 6.2, 6-H), 7.22 (5H, m, Ph); δ_C ($CDCl_3$) 18.03 (*i*-Pr Me), 19.36 (*i*-Pr Me), 25.40 (C-2), 26.40 (Ac Me), 32.86 (C-9), 38.66 (C-1), 48.62 (C-3), 53.00 (MeO), 58.03 (C-6), 126.56 (Ph), 127.50 (Ph), 128.80 (Ph), 135.19 (C-5), 163.05 (C=O), 171.26 (Ac C=O).

A second product was obtained (**233**), (1.46g, 32%) corresponding to the N-methylated product (previously characterised).

Direct diazomethane addition in the dark

To a solution of the (Z)- benzylidene (**227Z**) (3g, 10.5mmol) in ether ($10cm^3$) was added ethereal diazomethane (105mmol, 10mol eq, method 1, page 156) with stirring at room temperature in the absence of light. After 2 days the excess diazomethane was quenched by the addition of glacial acetic acid. The solution was reduced *in vacuo* and the residue was purified by flash chromatography, to yield a 3:1 mix of diasteromeric O-methylated cyclopropanes (**235**) (0.75g, 23%). Previously characterised.

Attempted carbene insertion using a silica gel template

To a solution of the (Z)-benzylidene (**227Z**) (100mg, 0.35mmol) in ether (10cm^3) was added pre-dried silica gel (2.9g, 190°C) and sodium bicarbonate (100mg, 1.2mmol, 3.5mol eq). To this was added ethereal diazomethane (3.5mmol, 10mol eq, method 2, page 157) at room temperature with stirring. Instantaneously the yellow colouration of the diazomethane disappeared. The silica was filtered off and the solution was worked up as normal. No cyclopropane product was isolated, just unreacted starting material (95mg, 95%).

Catalysed cyclopropanations

i. To a solution of the (Z)-benzylidene (**227Z**) (100mg, 0.35mmol) in ether (10cm^3) was added palladium (II) acetate (8mg, 0.035mmol) followed by an ethereal solution of diazomethane (3.5mmol, 10mol eq, method 1, page 156) with stirring at room temperature. After stirring for 15h the diazomethane was quenched with glacial acetic acid. The palladium salts were then filtered off and the filtrate was worked up as usual. The residue was purified by flash chromatography to yield a 4:1 mix of diastereomeric O-methylated cyclopropanes (**235**) (30mg, 27%). Previously characterised.

ii. To a solution of the (Z)-benzylidene (**227Z**) (100mg, 0.35mmol) in ether (10cm^3) was added palladium(II) chloride (6mg, 0.035mmol) followed by an ethereal solution of diazomethane (3.5mmol, 10mol eq, method 1, page 156) with stirring at room temperature. After stirring for 12h, the excess diazomethane was removed in the usual manner, the palladium salts filtered, and the filtrate worked up as normal. The residue was purified by flash chromatography yielding a 3.5:1 mix of diastereomeric O-methylated cyclopropanes (**235**) (25mg, 23%). Previously

characterised.

Preparation of (6S)-1,4-diacetyl-3-benzylidene-6-iso-propylpiperazin-2,5-dione (**241**)

To a solution of the (Z)-benzylidene (**227Z**) (2g, 7mmol) in acetic anhydride (20cm^3) was added pyridine (0.6cm^3, 7mmol) with stirring at room temperature. The solution was heated to 70°C for 7h, the solvent removed *in vacuo* and the residue purified by flash chromatography using ethyl acetate-petrol (15:85) as eluant. The desired product was isolated as a white solid (1.2g, 52%) m.p.158-9°C, (R_f=0.37, ethyl acetate-petrol (2:8)); (Found C, 66.0; H, 6.19; N, 8.61. $C_{18}H_{20}N_2O_4$ requires C, 65.85; H, 6.10; N, 8.54). δ_H ($CDCl_3$) 0.96 (3H, d, *J* 6, *i*-Pr Me), 1.17 (3H, d, *J* 6, *i*-Pr-Me), 2.19 (1H, m, *i*-Pr CH), 2.47 (3H, s, N-Ac), 2.58 (3H, s, N-Ac), 5.09 (1H, d, *J* 11, 6-H), 7.38 (5H, m, Ar), 7.57 (1H, s, vinyl H); δ_C ($CDCl_3$) 19.20 (*i*-Pr Me), 19.53 (*i*-Pr Me), 25.95 (Ac Me), 27.47 (Ac Me), 31.46 (C-7), 65.20 (C-6), 124.36 (C-3), 128.67 (Ph),128.96 (Ph), 130.23 (Ph), 133.15 (Ph), 134.22 (C-9), 163.93 (CONH), 167.53 (CONH), 168.37 (Ac C=O), 171.13 (Ac C=O); *m/z* (C.I.) 329 (MH^+, 100%), 287 (MH^+-Ac, 95%).

Attempted preparation of (Z)-(6S)-1-acetyl-3-benzylidene-6-iso-propyl-4-trimethylsilylpiperazin-2,5-dione (**244Z**)

To a solution of (Z)-benzylidene (**227Z**) (100mg, 0.35mmol) in dry THF (3cm^3) was added a solution of potassium *t*-butoxide (43mg, 0.38mmol) in THF (3cm^3) of 0°C with stirring. To the yellow solution that formed was added neat trimethylsilyl chloride (44µl, 0.35mmol). After stirring for 1 day the solution was worked up by pouring onto ice and extracted into ethyl acetate (3x10cm^3). The combined organic extracts were dried over anhydrous sodium sulphate and the solvent reduced *in vacuo*. The residue was purified by flash chromatography on silica gel using ethyl acetate-petrol (3:7) as eluant. Only starting material (**227Z**) was recovered (60mg, 60%).

Attempted cyclopropane construction via the additon of diazomethane to the bis-acetylated benzylidene (**241**)

To a solution of the bis-acylated benzylidene (**241**) (0.5g, 1.5mmol) in ether ($10cm^3$) was added an ethereal diazomethane solution (15mmol, 10mol eq, method 1, page 156) with stirring at room temperature in the presence of day light. After 1 day the excess diazomethane was quenched with glacial acetic acid and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel using ethyl acetate-petrol (6:94) as eluant; to yield an inseparable mix of the desired product (**242**) and the vinyl methyl adduct (**243**) (1:1.5) in 80mg (13%) yield.

Attempted cyclopropane construction via the addition of a sulphoxonium ylide to the bis-acetylated benzylidene (**241**).

To a $50cm^3$ one-neck rbf containing trimethylsulphoxonium iodide (242mg, 1.1mmol) and sodium hydride (26mg, 1.1mmol) was added DMSO ($10cm^3$) whilst stirring under argon at 0°C. The mixture was then stirred until all the trimethylsulphoxonium iodide had disappeared. Then a solution of bis-acetylated benzylidene (**241**) (0.33g, 1mmol) in DMSO ($2cm^3$) was added dropwise and the reaction was followed by tlc. After 5h at room temperature, 10h at 80°C and 1h at 100°C, the reaction mixture was poured into water ($80cm^3$), extracted with ether ($3x20cm^3$) and the collected organic fractions were dried over anhydrous Na_2SO_4. After filtration the solvent was reduced *in vacuo*. A multi component mixture was obtained from which none of the desired compound could be isolated.

(Z)-(1S,3S,6S)-7(H)-6-iso-propyl-5-methoxy-1-phenyl-4,7-diazaspiro[2,5]oct-4-en-8-one (**251**)

To a solution of (**237**) (0.67g, 2.1mmol) in methanol ($20cm^3$) was added potassium

carbonate (0.29g, 2.1mol) with stirring at 0°C. After 0.5h the solvent was removed *in vacuo*. The residue was taken up in DCM ($20cm^3$) and the potassium salts were removed *via* filtration. The filtrate was reduced *in vacuo*, to yield a colourless oil (0.57g, 100%) (R_f=0.12 ethyl acetate-petrol (2:8)); $[\alpha]^{20}_D$ -260°(c 1.63 in $CHCl_3$); υ_{max} ($CHCl_3$) 3390 (NH), 1650 (C=N); δ_H ($CDCl_3$) 0.68 (3H, d, *J* 6.8, *i*-Pr Me), 0.92 (3H, d, *J* 7.0, *i*-Pr Me), 1.65 (1H, dd, J_{22} 4.7, $J_{2B,1}$ 8.0, 2B-H), 2.03 (1H, m, *i*-Pr CH), 2.19 (1H, dd, J_{22} 4.7, $J_{2A,1}$ 9.5, 2A-H), 2.86 (1H, dd, $J_{1,2B}$ 8.0, $J_{1,2A}$ 9.5, 1-H), 3.43 (3H, s, MeO), 3.99 (1H, d, *J* 5.5, 6-H), 7.16-7.27 (5H, m, Ph), 7.62 (1H, s, NH); δ_C ($CDCl_3$) 15.86 (*i*-Pr Me), 18.33 (*i*-Pr Me), 23.32 (C-2), 31.88 (C-9), 35.19 (C-1), 45.51 (C-3), 53.00 (MeO), 58.00 (C-6), 126.01 (Ph), 127.34 (Ph), 128.93 (Ph), 136.29 (Ph), 159.45 (C-5), 171.74 (CONH); *m/z*(E.I.) 272 (M^+, 272.15765 $C_{16}H_{20}N_2O_2$ requires 272.15248 29%), 229 (160, M-C_3H_7).

(Z)-(1S, 3S, 6S)-6-iso-propyl-5,8-dimethoxy-1-phenyl-4,7-diazospiro[2,5] octa-4,7-diene (**252**)

To a solution of (**251**) (1.22g, 4.5mmol) in dry DCM ($30cm^3$) was added trimethyloxoniumtetrafluoroborate (2.0g, 13.5mmol) with stirring at 0°C. After 2 days the reaction mix was washed with phosphate buffer solution (sodium phosphate, monobasic (21.6g, 0.2mmol) and sodium phosphate, dibasic (85.2g, 0.5mmol) in water ($500cm^3$) and extracted into DCM ($3x30cm^3$). The combined organic extracts were dried over anhydrous sodium sulphate, filtered, and reduced *in vacuo*. The residue was purified *via* column chromatography on silica gel using ethyl acetate-petrol (1:9) as eluant. The desired product (**252**) (0.91g, 71%) was isolated as a colourless oil. (R_f=0.72 ethyl acetate-petrol): $[\alpha]^{20}_D$ -185°(c 1.62 in $CHCl_3$); υ_{max} ($CHCl_3$) 1655 vs (C=N), 1435 and 1320; δ_H ($CDCl_3$) 0.58 (3H, d, *J* 6.8, *i*-Pr Me), 0.94 (3H, d, *J* 7.0, *i*-Pr Me), 1.55 (1H, dd, J_{22} 5.0, $J_{2B,1}$ 7.7, 2B-H), 2.05 (1H, dd, J_{22} 5.0, $J_{2A,1}$ 9.5, 2A-H), 2.06 (1H, m, *i*-Pr CH), 2.70 (1H, dd, $J_{1,2B}$ 7.7, $J_{1,2A}$ 9.5, 1-H), 3.41 (3H, s, MeO), 3.71

(3H, s, MeO), 4.12 (1H, d, $J_{6,9}$ 3.3, 6-H), 7.11-7.23 (5H, m, Ph); δ_C ($CDCl_3$) 16.83 (*i*-Pr Me), 19.04 (*i*-Pr Me), 21.83 (C-2), 31.98 (C-9), 32.66 (C-1), 44.11 (C-3), 52.35 (MeO), 52.67 (MeO), 61.79 (C-6), 125.72 (Ph), 127.24 (Ph), 128.93 (Ph), 136.81 (Ph), 163.34 (C-8), 164.32 (C-5); *m/z* (E.I.) 286 (M^+, 286.16268 $C_{17}H_{22}N_2O_2$ requires 286.16813, 11%), 243 (100, M-C_3H_7).

*(Z)-(2S, 3S)-2,3-methanophenylalanine methyl ester((2S,3S) ∇^ZPhe-OMe) (**253**)*

To neat bis imine ether (**252**) (0.86g, 3mmol) was added 0.25M hydrochloric acid (24cm^3, 6mmol) with stirring at RT. After 1 day ether (5cm^3) was added and stirred for a further 24h. The reaction mix was partitioned between ether (5cm^3) and water (5cm^3) to remove any unreacted **252**. The aqueous phase was basified to pH 9 with 33% w/w aqueous ammonia solution and extracted with ether (3x30cm^3). The combined organic extracts were dried over anhydrous sodium sulphate and reduced *in vacuo*. The crude residue was purified by column chromatography on silica gel using ethyl acetate-petrol (4:6) as eluant. The title compound (**253**) was isolated as a colourless oil (0.33g, 58%) (R_f=0.15 ethyl acetate-petrol (2:8)) (Found C, 69.30; H, 7.10; N, 7.25. $C_{11}H_{13}NO_2$ requires C, 69.11; H, 6.81; N, 7.33); υ_{max} ($CHCl_3$) 3510 and 3385 (NH_2), 1710 (ester C=O); δ_H ($CDCl_3$) 1.43 (1H, dd, J_{22} 4.9, $J_{2B,3}$ 7.6, 2B-H), 1.56 (2H, s, 2xNH), 1.82 (1H, dd, J_{22} 4.9, $J_{2A,3}$ 9.5, 2A-H), 2.81 (1H, dd, $J_{3,2B}$ 7.6, $J_{3,2A}$ 9.5, 3-H), 3.75 (3H, s, MeO), 7.20-7.34 (5H, m, Ph); δ_C ($CDCl_3$) 21.93 (C-2'), 33.05 (C-3), 40.90 (C-2), 52.28 (MeO), 126.69 (Ph), 128.02 (Ph), 129.06 (Ph), 135.55 (Ph), 175.73 (C=O). *m/z* (E.I.) 191 (M^+, 8%), 176 (13, M-CH_3), 159 (72, M-CH_3OH), 132 (92, M-CO_2Me).

*(Z)-(2S,3S)-N-(tosylamino)methanophenylalanine methyl ester (**254**)*

To a solution of ∇^ZPhe-OMe (**253**) (47mg, 0.25mmol) in dry THF (4cm^3) was added a solution of tosyl chloride (47mg, 0.25mmol) in THF (4cm^3) and neat triethylamine (34µl, 0.25mmol) with stirring at RT. The reaction mixture was refluxed for 2h and the

solvent was then removed *in vacuo*. The residue was purified by column chromatography on silica gel using ethyl acetate-petrol (1:9) to yield a colourless crystalline solid (67mg, 78%) m.p.141-2°C; (R_f=0.38 ethyl acetate-petrol (3:7)); (Found C, 62.30; H, 5.50; N, 3.94. $C_{18}H_{19}NO_4S$ requires C, 62.58; H, 5.50; N, 4.06); $[\alpha]^{22}_D$ -47° (c 3.05 in $CHCl_3$); υ_{max} ($CHCl_3$) 3271 (NH), 1729 vs (CO_2Me), 1333 (SO_2NH); δ_H ($CDCl_3$) 2.14 (1H, dd, J_{22} 6.3, $J_{2A,3}$ 9.8, 2A-H), 2.24 (1H, dd, J_{22} 6.3, $J_{2B,3}$ 8.0, 2B-H), 2.43 (3H, s, Tosyl Me), 2.67 (1H, dd, $J_{3,2B}$ 8.0, $J_{3,2A}$ 9.8, 3-H), 3.41 (3H, s, MeO), 4.85 (1H, s, NH), 7.08-7.11 (2H, m, 2x *o*-Tosyl), 7.26-7.31 (5H, m, Ph), 7.69 (2H, d, *J* 8.3, 2x *m*-Tosyl); δ_C ($CDCl_3$) 18.42 (C-2'), 21.54 (Me), 34.12 (C-3), 41.13 (C-2), 52.51 (MeO), 127.53 (Ph), 128.28 (Ph), 128.83 (Ph), 129.42 (Ph), 133.34 (Ph), 137.07 (Ph), 143.65 (Ph),171.65 (C=O); *m/z* (C.I., *iso*-butane) 346 (MH^+, 100%), 326 (8), 314 (5, M-CH_4O), 286 (30).

(E)-(1R, 3S,6S)-N(7)-acetyl-6-iso-propyl-5-methoxy-1-phenyl-4,7-diazaspiro[2,5]oct-4-en-8-one (**256**)

To a solution of (E)-benzylidene (**227E**) (1.6g, 5.6mmol) in dry ether (20cm^3) was added a freshly distilled ethereal solution of diazomethane (56mmol, 10mol eq, method 1, page 156) with stirring, under nitrogen at room temperature. After 2 days, the excess diazomethane was removed *via* the addition of glacial acetic acid (3cm^3). The solvent was removed *in vacuo* and the residue was purified *via* column chromatography on silica gel using ethyl acetate-petrol (6:94) as eluant. The desired product (**256**) was isolated as a colourless oil (0.24g, 14%) (R_f=0.59 ethyl acetate-petrol (2:8)); $[\alpha]^{22}_D$ +170° (c 3.0 in $CHCl_3$); υ_{max} ($CHCl_3$) 1697 (amide C=O, acyl C=O and C=N), 1215 (C-N); δ_H ($CDCl_3$), 0.74 (3H, d, *J* 7.0, *i*-Pr Me), 0.97 (3H, d, *J* 7.0, *i*-Pr Me), 1.83 (1H, dd, J_{22} 4.5, $J_{2B,1}$ 9.2, 2B-H), 1.89 (1H, m, *i*-Pr CH), 2.40 (3H, s, N-Ac), 2.50 (1H, dd, J_{22} 4.5, $J_{2A,1}$ 9.2, 2A-H), 2.77 (1H, *t*, $J_{1,2B}$ 9.2, $J_{1,2A}$ 9.2, 1-H), 3.74 (3H, s, MeO), 4.86 (1H, d, $J_{6,9}$ 7.9, 6-H), 7.26 (5H, m, Ph); δ_C ($CDCl_3$) 19.17 (2 x *i*-Pr Me), 23.94 (C-2),

26.56 (Ac Me), 32.95 (C-9), 40.19 (C-1), 50.73 (C-3), 53.32 (MeO), 58.25 (C-6), 126.95 (Ph), 127.79 (Ph), 129.09 (Ph), 133.73 (Ph), 164.71 (C-5), 169.25 (C-8), 171.58 (Ac C=O); *m/z* (E.I.) 314 (M^+, 314.16643 $C_{18}H_{22}N_2O_3$ requires 314.16304, 10%), 272 (8, M-CH_2CO), 229 (66, 272-C_3H_7).

(E)-(1R,3S,6S)-7(H)-6-iso-propyl-5-methoxy-1-phenyl-4,7-diazaspiro[2,5]oct-4-en-8-one (**257**)

To a solution of (**256**) (0.24g, 0.76mmol) in DCM (20cm^3) was added potassium carbonate (0.12g, 0.84mmol) with stirring at 0°C. After stirring for 4h the potassium salts were removed *via* filtration. The filtrate was reduced *in vacuo* to yield a pale yellow oil (0.21g, 100%) (R_f=0.08 ethyl acetate-petrol (2:8)); $[\alpha]^{20}_D$ +227°(c 1.85 in $CHCl_3$); υ_{max} ($CHCl_3$) 3412 (NH), 1668 (amide C=O and C=N), 1224 (C-N); δ_H ($CDCl_3$) 0.81 (3H, d, *J* 4.0, *i*-Pr Me), 0.83 (3H, d, *J* 4.0, *i*-Pr Me), 1.61 (1H, dd, $J_{2B,1}$ 8.9, J_{22} 4.5, 2B-H), 2.05 (1H, m, *i*-Pr CH), 2.35 (1H, dd, $J_{2A,1}$ 8.9, J_{22} 4.5, 2A-H), 2.64 (1H, t, $J_{1,2A}$ 8.9, $J_{1,2B}$ 8.9, 1-H), 3.70 (3H, s, MeO), 3.93 (1H, t, $J_{6,9}$ 7.0, 6-H), 6.39 (1H, s, NH), 7.14-7.55 (5H, m, Ph); δ_C ($CDCl_3$) 16.44 (*i*-Pr Me), 18.36 (*i*-Pr Me), 21.96 (C-2), 31.72 (C-9), 37.98 (C-1), 47.29 (C-3), 53.19 (MeO), 59.36 (C-6), 126.37 (Ph), 127.53 (Ph), 129.22 (Ph), 135.09 (Ph), 160.00 (C-5), 168.05 (C-8); *m/z* (E.I.) 272 (M^+, 12%), 229 (62, M-C_3H_7), 43 (30), 28 (100).

(E)-(1R,3S,6S)-6-iso-propyl-5,8-dimethoxy-1-phenyl-4,7-diazaspiro[2,5]octa-4,7-diene (**258**)

To a solution of (**257**) (0.21g, 0.76mmol) in DCM (15cm^3) was added trimethyloxoniumtetrafluoroborate (0.23g, 1.5mmol) with stirring at RT. After 2 days the reaction mix was washed with phosphate buffer solution and extracted into DCM (3x15cm^3). The combined organic extracts were dried over anhydrous sodium sulphate, filtered, and reduced *in vacuo*. The residue was purified *via* column

chromatography on silica gel using ethyl acetate-petrol (1:9) as eluent. The desired product (**258**) was isolated as a pale yellow oil (140mg, 65%) (R_f=0.64 ethyl acetate-petrol (2:8); $[\alpha]^{20}_D$ +259°(c 2.02 in $CHCl_3$); υ_{max} ($CHCl_3$) 1670 (C=N), 1218 (C-N and C-O); δ_H ($CDCl_3$) 0.82 (3H, d, *J* 6.8, *i*-Pr Me), 1.00 (3H, d, *J* 7.0, *i*-Pr Me), 1.53 (1H, dd, J_{22} 5.0, $J_{2B,1}$ 9.4, 2B-H), 2.13 (1H, m, *i*-Pr CH), 2.21 (1H, dd, J_{22} 5.0, $J_{2A,1}$ 9.4, 2A-H), 2.54 (1H, t, $J_{1,2A}$ 9.4, $J_{1,2B}$ 9.4, 1-H), 3.22 (3H, s, MeO), 3.68 (3H, s, MeO), 4.11 (1H, d, $J_{6,9}$ 4.0, 6-H), 7.16-7.25 (5H, m, Ph); δ_c ($CDCl_3$) 17.71 (*i*-Pr Me), 19.20 (*i*-Pr Me), 20.21 (C-2), 32.17 (C-9), 35.65 (C-1), 45.90 (C-3), 51.70 (MeO), 52.64 (MeO), 62.44 (C-6), 125.98 (Ph), 127.44 (Ph), 129.06 (Ph), 136.32 (Ph), 163.15 (C-8), 166.30 (C-5); *m/z* (E.I.) 286 (M^+, 286.16540 $C_{17}H_{22}N_2O_2$ requires 286.16813, 3%) 243 (100, M-C_3H_7), 228 (15), 105 (12).

(E)-(2S,3R)-2,3-methanophenylalanine methyl ester((2S,3R) ∇^E*Phe-OMe) (***259**)

To neat bis imine ether (**258**) (260mg, 0.91mmol) was added 0.25M hydrochloric acid (7.3cm^3, 1.82mmol) with stirring at room temperature. After 1 day, ether (5cm^3) was added and stirred for a further 24h. The reaction mix was partitioned between ether (5cm^3) and water (5cm^3), the aqueous phase was basified to pH8 with 1M ammonium hydroxide and extracted with ether (3x15cm^3). The combined organic extracts were dried over anhydrous sodium sulphate and reduced *in vacuo*. The crude residue was purified by column chromatography on silica gel using ethyl acetate-petrol (4:6) as eluant. The title compound (**259**) was isolated as a pale yellow oil (39mg, 22%) (R_f=0.26 ethyl acetate-petrol (3:7)); υ_{max} ($CHCl_3$) 3380 (NH_2), 1710 (CO_2Me), 1215 (C-N); δ_H ($CDCl_3$) 1.47 (1H, dd, J_{22} 5.3, $J_{2B,1}$ 8.6, 2B-H), 1.99 (1H, dd, J_{22} 5.3, $J_{2A,1}$ 8.6, 2A-H), 2.43 (2H, s, NH_2), 2.67 (1H, t, $J_{1,2A}$ 8.6, $J_{1,2B}$ 8.6, 1-H), 3.30 (3H, s, MeO), 7.22-7.34 (5H, m, Ph); δ_c ($CDCl_3$) 19.98 (C-2'), 36.26 (C-3), 43.20 (C-2), 51.51 (MeO), 126.56 (Ph), 127.86 (Ph), 128.99 (Ph), 136.39 (Ph), 173.69 (C=O); *m/z* (E.I.) 191 (M^+, 4%),. 190 (M-H, 190.08665 $C_{11}H_{12}NO_2$ requires 190.08680, 3%), 176 (27, M-CH_3),

159 (68,M-CH_3OH), 132 (94,M-CO_2Me), 115 (34).

Coupling of (Z)- and (E)-2,3-methanophenylalanine methyl ester (253) and (259) with an N-protected trytophan (206).

Preparation of N^{α}-(2-adamantyloxycarbonyl)-α-methyl-R-tryptophanyl-(Z)-(2S,3S)-2,3-methanophenylalanine (**260**)

Method 1.

To a solution of 2-Adoc-R-α-Me-Trp-OH (**206**) (104mg, 0.26mmol) in ethyl acetate ($5cm^3$) was added HOBt (44mg, 0.29mmol) in solid form followed by a solution of DCC (65mg, 0.31mmol) in ethyl acetate ($2cm^3$) with stirring at room temperature. After 1h the precipitated dicyclohexylurea (DCU) was filtered and to the filtrate was added a solution of(Z)-(2S,3S)-2,3-methanophenylalanine methyl ester (**253**) (50mg, 0.26mmol) in ethyl acetate ($1cm^3$) at 0°C. After 4h the solution was brought to reflux. The solution was worked up after a further 6h by washing with water and extracting into ethyl acetate ($3x10cm^3$). The combined organic extracts were dried over sodium sulphate and reduced *in vacuo* to yield a pale yellow oil. The residue was purified by flash chromatography to yield no desired dipeptide (**260**). Only the starting material (**253**) (40mg, 80%) was recovered.

Method 2.

To a solution of 2-Adoc-R-α-Me-Trp-OH (247mg, 0.62mmol) in dry THF ($5cm^3$) was added NMM (68µl, 0.62mmol) and *iso*-butyl chloroformate (81µl, 0.62mmol) successively with stirring at RT. After 30 min a solution of (2S, 3S) ∇^{Z}Phe-OMe (**253**) (100mg, 0.52mmol) in dry THF ($5cm^3$) was added and stirred for 1 day. The solvent was then worked up by washing with water ($5cm^3$) extracting into DCM ($3x5cm^3$) and

drying the combined organic extracts over anhydrous sodium sulphate. The solvent removed *in vacuo* and the residue was purified by column chromatography on silica gel by using ethyl acetate-petrol (1:9) as eluant. The title compound (**260**) was isolated as a yellow foam (118mg, 40%) (R_f=0.13 ethyl acetate-petrol (3:7); $[\alpha]^{20}_D$ +6° (c 1.35 in $CHCl_3$); υ_{max} ($CHCl_3$) 3418 (amide NH), 1718 vs (CO_2Me), 1686 vs (CONH), 1491 (Ar. C=C), 1215 (C-N); δ_H ($CDCl_3$) 1.39 (3H, s, R-α-Me), 1.44-1.93 (15H, m, adamantyl and 2'B-H.2), 2.18 (1H, dd, $J_{2',2'}$ 5.8, $J_{2'A,3}$ 9.8, 2'A-H.2), 2.95 (1H, dd, $J_{3,2'B}$ 8.0, $J_{3,2'A}$ 9.8, 3-H.2), 3.13 (1H, d, J_{gem} 15, 3-H.1) 3.37 (1H, d, J_{gem} 15, 3-H.1), 3.73 (3H, s, MeO), 4.61 (1H, s, adamantyl 2-H), 5.02 (1H, s, 2-NH.1), 6.87-7.56 (11H, m, Ph and indole Ar, 2-NH.2), 8.29 (1H, s, indole NH); δ_c ($CDCl_3$) 14.17 (R-α-Me), 20.98 (C-2'.2), 23.09 (Ad), 26.92 (Ad), 27.08 (Ad), 31.62 (C-3.1), 31.88 (Ad), 31.95 (Ad), 32.14 (Ad), 32.37 (C-3.2), 36.29 (Ad), 37.30 (Ad), 39.08 (Ad), 52.64 (C-2.2), 60.39 (MeO), 60.72 (C-2.1), 109.76 (Ar), 111.09 (Ar), 119.10 (Ar), 119.65 (Ar), 122.02 (Ar), 123.90 (Ar), 127.18 (Ar), 128.25 (Ar), 128.77 (Ar), 134.18 (Ar), 135.87 (Ar), 155.27 (C=O), 172.13 (OC=O), 175.70 (CO_2Me); *m/z* (E.I.) 569 (M^+, 5%), 440 (5), 417 (6), 395 (2), 379 (3), 130 (100).

Method 3.

To a solution of 2-Adoc-R-α-Me-Tro-OH (**206**) (125mg, 0.3mmol) in dry THF ($3cm^3$) was added NMM (35µl, 0.3mmol), HOBt (48mg, 0.3mmol) and EDC (60mg, 0.3mmol) successively with stirring at 0°C for 1 hour. To this was added a solution of (2S,3S) ∇^ZPhe-OMe (**253**) (60mg, 0.3mmol) in dry THF ($3cm^3$). After stirring for 3 days at room temperature, no desired product was observed. The solution was brought to reflux. After 3h no starting material was detected by tlc. The solvent was removed *in vacuo* and the residue taken up in ethyl acetate ($5cm^3$) and washed with water ($5cm^3$). The organic layer was dried over sodium sulphate and reduced *in vacuo*. The remaining residue was purified *via* column chromatography on silica gel using ethyl

acetate-petrol (2:8) as eluant. The desired product (**260**) was isolated as a pale yellow foam (53mg, 31%). Previously characterised.

Preparation of N^{α}-(2-adamantyloxycarbonyl)-α-methyl-R-trypophanyl-(E)-(2S,3R)-2,3-methanophenylalanine methyl ester (**262**)

To a solution of 2-Adoc-R-α-Me-Trp-OH (**206**) (162mg, 0.41mmol) in dry THF ($10cm^3$) was added successively NMM (45μl, 0.41mmol) and then *iso*-butyl chloroformate (54μl, 0.42mmol) with stirring at 0°C. After 30min a solution of (2S,3R) ∇^{E}Phe-OMe (**259**) (52mg, 0.27mmol) in THF ($5cm^3$) was added. After stirring for 1 day the reaction was worked up in the usual manner and the residue was purified by column chromatography on silica by using ethyl acetate-petrol (2:8). The title compound (**262**) was isolated as a pale yellow foam (74mg, 32%); $[\alpha]^{22}_{D}$ +68° (c 2.92 in $CHCl_3$); υ_{max} ($CHCl_3$) 3390 (amide NH), 1717 (CO_2Me), 1686 (CONH), 1492 (Ar C=C); δ_H ($CDCl_3$,) 1.13-2.01 (18H, m, adamantyl, R-α-Me and 2'B-H.2), 2.18 (1H, dd, $J_{2'2'}$ 5.7, $J_{2'A,3}$ 8.6, 2'A-H.2), 2.81 (1H, dd, $J_{3,2'A}$ 9.7, $J_{3,2'B}$ 8.6, 3-H.2), 3.32 (3H, s, CO_2Me), 3.41 (1H, d, J_{gem} 14.8, 3-H.1), 3.61 (1H, d, J_{gem} 14.8, 3-H.1), 4.85 (1H, br s, adamantyl 2-H), 5.24 (1H, br s, 2-NH.1), 7.08-7.66 (11H, m, Ph and indole Ar, 2-NH.2), 8.32 (1H, br s, indole NH); δ_C ($CDCl_3$) 15.05 (R-α-Me), 20.40 (C-2'.2), 24.10 (Ad), 24.78 (Ad), 25.43 (Ad), 26.89 (Ad), 27.12 (Ad), 29.68 (Ad), 31.49 (C-3.1), 31.75 (Ad), 32.14 (Ad), 33.60 (Ad), 34.96 (C-3.2), 36.29 (Ad), 37.30 (Ad), 49.30 (C-2.2), 51.86 (OMe), 60.75 (C-2.1), 109.99 (Ar), 111.19 (Ar), 118.94 (Ar), 119.72 (Ar), 122.05 (Ar), 124.03 (Ar), 126.92 (Ar), 127.89 (Ar), 128.41 (Ar), 129.32 (Ar), 135.35 (Ar), 135.96 (Ar), 155.08 (C=O), 169.99 (OC=O), 175.34 (CO_2Me); *m/z* (+FAB) 570 (MH^+, 52%), 379 (12), 225 (36), 135 (100).

*Hydrolysis of the (Z)- dipeptide ester (**260**)*

Method 1.

To a solution of the dipeptide (**260**) (26mg, 0.046mmol) in THF ($2cm^3$) was added 0.1M lithium hydroxide solution (aq) ($0.5cm^3$, 0.05mmol) with stirring at RT. After 2h no change had been observed and so the solution was refluxed for a further 3h the reaction was then worked up by washing with 0.1M hydrochloric acid ($5cm^3$) and extracted into ethyl acetate ($2x5cm^3$). The combined organic extracts were dried over anhydrous sodium sulphate and reduced *in vacuo*. No dipeptide acid was isolated, only starting material (**260**) was recovered (22mg, 85%).

Method 2.

To a solution of the dipeptide ester (**260**) (58mg, 0.1mmol) in ethanol ($2cm^3$) was added 0.1M sodium hydroxide solution (aq) ($1.1cm^3$, 0.11mmol) with stirring at RT. After 1h no change had been observed. The solution was refluxed for 2h when no starting material was detected by tlc. The reaction was worked up by washing with 0.1M hydrochloric acid ($10cm^3$) and extracted into ethyl acetate ($2x10cm^3$). The combined organic extracts were dried over anhydrous sodium sulphate, and reduced *in vacuo*. This generated the desired carboxylic acid (**266**) (50 mg, 88%) as a colourless oil (R_f = 0.49 methanol-DCM (1:9)). The product was further purified *via* reverse-phase column chromatography using methanol-water (3:1) as eluant. This yielded a white foam. $[\alpha]^{22}_D$ +23° (c 0.77 in $CHCl_3$); υ_{max} 3405 (NH), 1695 (CO_2H, CONH), 1498 (Ar C=C), 1216 (C-N); δ_H ($CDCl_3$) 1.26 (3H, s, R-α-Me), 1.38-1.93 (15H, m, adamantyl and 2'B-H.2), 2.20 (1H, dd, $J_{2'2'}$ 6, $J_{2'A,3}$ 9, 2'A-H.2), 3.03 (1H, t, $J_{3,2'A}$ 9, $J_{3,2'B}$ 9, 3-H.2), 3.21 (2H, s, 3-H.1), 4.71 (1H, s, CO_2H), 5.12 (1H, s, adamantyl 2-H), 6.22 (1H, s, 2-NH.1), 6.88-7.50 (11H, m, Ph and indole Ar and 2-NH.2), 8.56 (1H, s, indole NH); δ_C ($CDCl_3$) 21.60 (C-2'.2), 22.90 (Ad), 26.82 (Ad),

27.05 (Ad), 31.62 (C-3.1), 31.88 (Ad), 32.63 (Ad), 32.95 (C-3.2), 36.20 (Ad), 37.23 (Ad), 60.62 (C-2.2), 108.92 (Ar), 111.35 (Ar), 118.87 (Ar), 119.72 (Ar), 122.02 (Ar), 124.65 (Ar), 127.40 (Ar), 128.15 (Ar), 128.38 (Ar), 128.60 (Ar), 133.76 (Ar), 136.03 (Ar), 155.95 (C=O), 173.24 (OC=O), 175.51 (CO_2Me); *m/z* 556 (M^+, 85%), 512 (3), 392 (8), 130 (100).

Attempted hydrolysis of the (Z)-imine ether (**237**) *to give the cyclopropyldipeptide* (**268**)

Method 1.

To the neat imine ether (**237**) (100mg, 0.32mmol) was added 0.25M hydrochloric acid (2.6cm^3, 0.64mmol) with stirring at room temperature. After 1 day, ether (3cm^3) was added and the solution was stirred for a further 2 hours. Tlc indicated that all the starting material had been converted into two products, both more polar than the starting material, one of which was baseline (ethyl acetate-petrol(1:1)). The reaction was worked up by washing with water (5cm^3) and extracting into DCM (3x5cm^3). The combined organic extracts were dried over anhydrous sodium sulphate and reduced *in vacuo* to give a colourless oil (70mg, 80%) corresponding to the deacylated cyclopropane (**251**)(previously characterised). Reverse-phase tlc on the remaining aqueous phase indicated an unseparable mixture of ninhydrin active components.

Method 2.

To a solution of the imine ether (**237**) (23mg, 0.07mmol) in THF (3cm^3) was added a 33% w/w aqueous ammonia solution (43µl, 0.7mmol) with stirring at room temperature. The reaction was monitored by tlc. After two days no baseline material was evident by tlc (ethyl acetate-petrol (1:1)). The solution was then worked up by

neutralising with 0.1M hydrochloric acid and extracting into DCM (3x5cm^3). The combined organic extracts were dried over anhydrous sodium sulphate to give an oil (20mg,10%). ^{1}H NMR indicated a 10:1 mix of deacylated cyclopropane (**251**) to starting material (**237**).

Preparation of the dipeptide (Z)-(2S,3S)-2,3-methanophenylalanyl-S-valine methyl ester (**268**)

To a solution of the (Z)-imine ether (**251**) (205mg, 0.75mmol) in acetonitrile (30cm^3) was added 0.1M hydrochloric acid (30cm^3, 3.0mmol) with stirring at room temperature. After stirring for 21h the solution was neutralised with sodium bicarbonate and extracted with DCM (3x20cm^3). The combined organic extracts were dried over anhydrous sodium sulphate and reduced *in vacuo*. The residue was purified by flash chromtography using ethyl acetate-petrol (3:7) as eluant to yield the desired compound (**268**) as a colourless oil (178mg, 82%). (R_f=0.28, ethyl acetate-petrol (3:7)); $[\alpha]^{22}_D$ -149° (c 3.13 in $CHCl_3$) υ_{max} ($CHCl_3$) 3377 br (amide NH and NH_2), 1739 (CO_2Me), 1665 (CONH), 1215 (C-N); δ_H ($CDCl_3$) 0.94 (3H, d, *J* 7.0, *i*-Pr Me), 0.98 (3H, d, *J* 7.0, *i*-Pr Me), 1.26 (1H, dd, $J_{2'2'}$ 5.0, $J_{2'B,3}$ 7.4, 2'B-H.1), 1.58 (2H, s, NH_2), 1.98 (1H, dd, $J_{2'2'}$ 5.0, $J_{2'A,3}$ 9.5, 2'A-H.1), 2.21 (1H, m, 3-H.2), 2.94 (1H, dd, $J_{3,2'B}$ 7.4, $J_{3,2'A}$ 9.5, 3-H.1), 3.75 (3H, s, MeO), 4.53 (1H, dd, $J_{2,2N}$ 5.1, $J_{2,3}$ 9.2, 2-H.2), 7.18-7.38 (5H, m, Ph), 8.30 (1H, d, $J_{2N,2}$ 9.2, 2-NH.2); δ_C ($CDCl_3$) 17.87 (C-4.2), 19.04 (C-4.2), 22.51 (C-2'.1), 31.23 (C-3.2), 32.56 (C-3.1), 40.28 (C-2.1), 51.99 (C-2.2), 57.47 (MeO), 127.05 (Ph), 128.60 (Ph), 129.03 (Ph), 135.58 (Ph), 172.68 (CONH), 174.47 (CO_2Me); *m/z* (+FAB) 291 (MH^+, 100%), 258 (3), 231 (10), 132 (70).

Preparation of the dipeptide (E)-(2S,3S)-2,3-methanophenylalanyl-S-valine methyl ester (**273**)

To a solution of the (E)-imine ether (**257**) (105mg, 0.39mmol) in acetonitrile ($15cm^3$) was added 0.1M hydrochloric acid ($15cm^3$, 1.5mmol) with stirring at room temperature. After stirring for 15h the solution was neutralised with sodium bicarbonate and extracted with DCM ($3x30cm^3$). The combined organic extracts wre dried over anhydrous sodium sulphate and reduced *in vacuo*. The residue was purified by flash chromatography using ethyl acetate-petrol (4:6) as eluant. This yielded the desired compound (**273**) as a colourless oil (89mg, 79%). (R_f=0.2 ethyl acetate-petrol (1:1)); (Found C, 66.00; H, 7.73; N, 9.30. $C_{16}H_{22}N_2O_3$ requires C, 66.21; H, 7.59; N, 9.66); υ_{max} ($CHCl_3$) 3370 br (amide NH and NH_2), 1736 (CO_2Me), 1665 (CONH); δ_H ($CDCl_3$), 0.65 (3H, d, *J* 6.8, *i*-Pr Me), 0.75 (3H, d, *J* 6.8, *i*-Pr Me), 1.31 (1H, dd, $J_{2',2}$ 5.1, $J_{2'B,3}$ 9.5, 2'B-H.1), 1.96 (1H, m, 3-H.2), 2.17 (3H, m, NH_2 and 2'A-H.1), 2.55 (1H, dd, $J_{3,2'B}$ 9.5, $J_{3,2'A}$ 8.1, 3-H.1), 3.68 (3H, s, MeO), 4.30 (1H, dd, J_{23} 4.9, $J_{2,2N}$ 9.1, 2-H.2), 7.22 (5H, m, Ph), 7.76 (1H, d, $J_{2N,2}$ 9.1, 2-NH.2); δ_C ($CDCl_3$) 17.51 (C-4.2), 18.62 (C-4.2), 21.34 (C-2'.1), 30.91 (C-3.2), 31.10 (C-3.1), 36.46 (C-2.1), 51.93 (C-2.2), 56.99 (MeO), 126.59 (Ph), 127.83 (Ph), 128.96 (Ph), 135.97 (Ph), 171.20 (CONH), 172.75 (CO_2Me); m/z (+FAB) 291 (MH^+, 49%) 231 (9), 132 (52), 109 (82).

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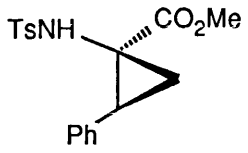
373 Highly toxic and carcinogenic. All apparatus should be handled using gloves and safety shield in a fume cupboard.

APPENDIX

X-ray crystallographic data for **254**



254

Compound **254** was crystallised from petrol-ethyl acetate.

A crystal of approximate dimensions 0.3 x 0.4 x 0.4 mm was used for data collection.

Crystal data: $C_{18}H_{19}O_4NS$, $M = 339.4$ monoclinic, $a = 10.335(1)$, $b = 9.066(2)$, $c = 10.371(2)$Å, $\beta = 109.94(1)$, $U = 913.5$ Å^3, space group $P2_1$, $Z = 2$, $D_c = 1.23$ gcm^{-3}, μ(Mo-K_α) = 1.50 cm^{-1}, $F(000) = 358$. Data were measured at room temperature on a Hilger and Watts Y290 four-circle diffractometer in the range $2 \leq \theta \leq 22^\circ$. 1171 reflections were collected of which 616 were unique with $I \geq 3\sigma(I)$. Data were corrected for Lorentz and polarization effects and the structure was solved by Direct methods and refined using the SHELX [1,2] suite of programs. In the final least squares cycles the sulphur and oxygen atoms along with carbons 11-12 were allowed to vibrate anisotropically. All other atoms were treated isotropically. Hydrogen atoms were included at calculated positions. Final residuals after 10 cycles of least squares were $R = R_w = 0.0876$, for unit weights. Max. final shift/esd was 0.010. The max. and min. residual densities were 0.13 and -0.14 eÅ^{-3} respectively. Final fractional atomic coordinates and anisotropic thermal parameters, bond distances and angles are given in Tables 16-17, 18 and 19-22 respectively. Tables of anisotropic temperature factors and hydrogen atom positions are available as supplementary data. The asymmetric unit is shown in Fig.10, along with the labelling scheme used.

1. Sheldrick G.M., SHELX86, a computer program for crystal structure determination, University of Göttingen, 1986.

2. Sheldrick G.M., SHELX76, a computer program for crystal structure determination, University of Cambridge, 1976.

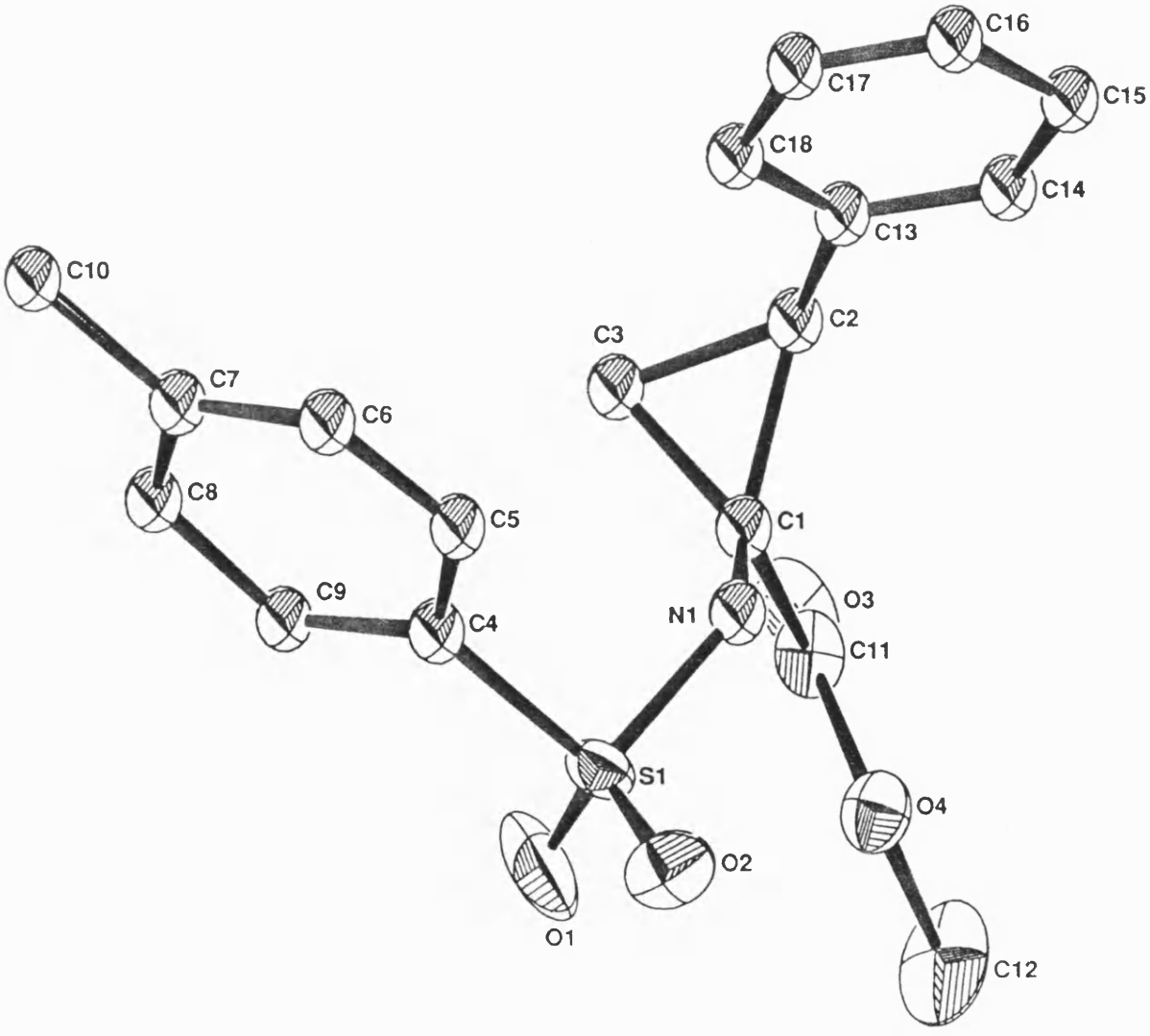


Fig 10. X-ray structure of (Z)-(2S,3S)-N-(tosylamino)-2,3-methanophenylalanine methyl ester (**254**).

Table 16 Fractional atomic coordinates and thermal parameters for **254** (Å)

Atom	x	y	z	Uiso or Ueq
S1	-0.7766(6)	-0.4666	-0.7164(6)	0.062(4)
O1	-0.8645(16)	-0.5514(39)	-0.6680(17)	0.150(22)
O2	-0.7528(18)	-0.3202(22)	-0.6823(17)	0.077(14)
O3	-0.6113(16)	-0.8810(30)	-0.4784(18)	0.079(14)
O4	-0.6102(15)	-0.6406(25)	-0.4251(16)	0.060(11)
C11	-0.6094(23)	-0.7524(40)	-0.5021(26)	0.053(17)
C12	-0.6181(32)	-0.6592(55)	-0.2898(23)	0.154(36)
N1	-0.6269(16)	-0.5506(24)	-0.6701(16)	0.039(4)
C1	-0.6057(20)	-0.6950(28)	-0.6435(23)	0.047(6)
C2	-0.4982(24)	-0.7771(36)	-0.6834(24)	0.069(8)
C3	-0.6518(23)	-0.8064(35)	-0.7543(24)	0.070(7)
C4	-0.8432(20)	-0.4800(36)	-0.8965(20)	0.057(6)
C5	-0.7832(22)	-0.4023(34)	-0.9766(22)	0.059(7)
C6	-0.8377(23)	-0.4021(38)	-1.1158(23)	0.071(7)
C7	-0.9453(21)	-0.5054(33)	-1.1781(22)	0.058(7)
C8	-1.0056(26)	-0.5804(35)	-1.1063(24)	0.074(8)
C9	-0.9544(21)	-0.5739(31)	-0.9603(21)	0.054(6)
C10	-1.0024(29)	-0.5144(43)	-1.3379(25)	0.102(10)
C13	-0.4173(21)	-0.6804(28)	-0.7536(23)	0.048(6)
C14	-0.2847(22)	-0.6462(34)	-0.6702(24)	0.065(7)
C15	-0.2019(26)	-0.5612(33)	-0.7183(24)	0.066(7)
C16	-0.2552(27)	-0.5045(38)	-0.8509(26)	0.091(9)
C17	-0.3835(25)	-0.5382(37)	-0.9297(27)	0.075(8)
C18	-0.4680(25)	-0.6299(35)	-0.8819(24)	0.070(7)

Table 17 Fractional atomic coordinates for the hydrogen atoms

Atom	x	y	z
H11	-0.6227	-0.6786	-0.5474
H21	-0.4081	-0.8445	-0.6374
H31	-0.6920	-0.9100	-0.7330
H32	-0.7067	-0.7742	-0.8591
H51	-0.6960	-0.3316	-0.9283
H61	-0.7956	-0.3353	-1.1782
H81	-1.0849	-0.6596	-1.1564
H91	-1.0049	-0.6332	-0.9006
H101	-0.9454	-0.4397	-1.3797
H102	-1.1100	-0.4845	-1.3755
H103	-0.9907	-0.6258	-1.3692
H121	-0.6177	-0.5517	-0.2448
H122	-0.5307	-0.7219	-0.2265
H123	-0.7119	-0.7164	-0.2969
H141	-0.2430	-0.6905	-0.5676
H151	-0.0990	-0.5323	-0.6531
H161	-0.1923	-0.4376	-0.8924
H171	-0.4227	-0.4975	-1.0338
H181	-0.5732	-0.6509	-0.9459

Table 18 Anisotropic thermal parameters (Å)

Atom	U11	U22	U33	U23	U13	U12
S1	0.052(3)	0.085(7)	0.051(3)	0.009(5)	0.019(3)	0.039(5)
O1	0.041(10)	0.340(45)	0.069(12)	0.062(18)	0.022(10)	0.038(16)
O2	0.115(16)	0.050(14)	0.067(12)	0.013(13)	0.028(11)	0.055(11)
O3	0.059(12)	0.078(16)	0.101(14)	0.027(15)	0.030(10)	0.005(12)
O4	0.081(12)	0.054(13)	0.046(9)	0.013(12)	0.021(8)	0.009(10)
C11	0.062(16)	0.036(17)	0.062(17)	0.022(17)	0.025(14)	-0.014(15)
C12	0.135(29)	0.299(62)	0.028(16)	0.035(26)	0.028(16)	-0.031(37)

Table 19 Bond lengths (Å)

S1	-O1	1.407(24)	S1	-O2	1.374(21)
S1	-N1	1.642(17)	S1	-C4	1.761(20)
O3	-C11	1.19(3)	O4	-C11	1.29(3)
O4	-C12	1.44(3)	N1	-C1	1.34(3)
C1	-C2	1.51(3)	C1	-C3	1.48(3)
C1	-C11	1.57(3)	C2	-C3	1.53(3)
C2	-C13	1.55(3)	C4	-C5	1.39(3)
C4	-C9	1.40(3)	C5	-C6	1.36(3)
C6	-C7	1.43(3)	C7	-C8	1.31(3)
C7	-C10	1.56(3)	C8	-C9	1.42(3)
C13	-C14	1.38(3)	C13	-C18	1.33(3)
C14	-C15	1.37(3)	C15	-C16	1.39(3)
C16	-C17	1.34(3)	C17	-C18	1.41(3)

Table 20 Bond angles (°)

O2	-S1	-O1	121(1)	N1	-S1	-O1	107(1)
N1	-S1	-O2	108(1)	C4	-S1	-O1	106(1)
C4	-S1	-O2	108(1)	C4	-S1	-N1	105(1)
C12	-O4	-C11	122(3)	C1	-N1	-S1	126(2)
C2	-C1	-N1	121(2)	C3	-C1	-N1	121(2)
C3	-C1	-C2	61(2)	C11	-C1	-N1	117(2)
C11	-C1	-C2	110(2)	C11	-C1	-C3	113(2)
C3	-C2	-C1	58(1)	C13	-C2	-C1	114(2)
C13	-C2	-C3	121(2)	C2	-C3	-C1	60(1)
C5	-C4	-S1	120(2)	C9	-C4	-S1	120(2)
C9	-C4	-C5	119(2)	C6	-C5	-C4	122(2)
C7	-C6	-C5	117(3)	C8	-C7	-C6	122(2)
C10	-C7	-C6	118(2)	C10	-C7	-C8	120(2)
C9	-C8	-C7	120(2)	C8	-C9	-C4	118(2)
O4	-C11	-O3	129(3)	C1	-C11	-O3	122(3)
C1	-C11	-O4	109(2)	C14	-C13	-C2	114(2)
C18	-C13	-C2	125(2)	C18	-C13	-C14	121(2)
C15	-C14	-C13	120(2)	C16	-C15	-C14	119(3)
C17	-C16	-C15	120(3)	C18	-C17	-C16	122(3)
C17	-C18	-C13	118(2)				

Table 21 Intermolecular distances (Å)

O1	...H151	2.49	1	1.0	0.0	0.0
O1	...H151	2.49	1	1.0	0.0	0.0
O2	...H81	2.41	2	-2.0	-1.0	-2.0
O2	...H141	2.86	2	-1.0	-1.0	-1.0
O3	...N1	2.87	2	-1.0	0.0	-1.0
O3	...H102	2.91	2	-2.0	0.0	-2.0
O4	...H21	2.75	2	-1.0	-1.0	-1.0
C2	...H121	2.97	2	-1.0	0.0	-1.0
H21	...C12	2.99	2	-1.0	0.0	-1.0
C6	...H91	2.97	2	-2.0	-1.0	-2.0
H61	...C14	2.64	2	-1.0	-1.0	-2.0
H61	...C15	2.70	2	-1.0	-1.0	-2.0

Table 22 Intramolecular distances (Å)

S1	...C1	2.66	S1	...H11	2.72
S1	...C5	2.74	S1	...H51	2.88
S1	...C9	2.75	S1	...H91	2.90
O1	...O2	2.42	O1	...N1	2.46
O1	...C1	2.91	O1	...H11	2.65
O1	...C4	2.54	O1	...C9	2.86
O1	...H91	2.47	O2	...N1	2.44
O2	...C4	2.55	O2	...H51	2.81
O3	...O4	2.25	O3	...C1	2.42
O3	...H11	1.96	O3	...C2	2.91
O3	...C3	2.83	O3	...H31	2.50
O3	...C12	2.82	O3	...H122	2.85
O3	...H123	2.86	O4	...N1	2.62
O4	...C1	2.33	O4	...H121	2.06
O4	...H122	2.08	O4	...H123	2.07
N1	...H11	1.71	N1	...C2	2.48
N1	...C3	2.46	N1	...H32	2.74
N1	...C4	2.71	N1	...C11	2.49
N1	...C13	2.85	C1	...H21	2.43
C1	...H31	2.21	C1	...H32	2.24
C1	...C13	2.57	H11	...C2	2.38
H11	...C3	2.37	H11	...C12	2.66
C2	...H31	2.24	C2	...H32	2.30
C2	...C11	2.53	C2	...C14	2.47
C2	...H141	2.62	C2	...C18	2.56
C2	...H181	2.81	H21	...C3	2.42

H21 ...C11	3.00	H21 ...C13	1.90
H21 ...C14	2.30	C3 ...C11	2.55
C3 ...C13	2.68	C3 ...H181	2.77
H31 ...C11	2.67	H32 ...C4	2.98
H32 ...C13	2.94	H32 ...C18	2.87
C4 ...H51	2.14	C4 ...C6	2.40
C4 ...C7	2.76	C4 ...C8	2.43
C4 ...H91	2.16	C5 ...H61	2.14
C5 ...C7	2.38	C5 ...C8	2.76
C5 ...C9	2.40	H51 ...C6	2.10
C6 ...C8	2.40	C6 ...C9	2.79
C6 ...C10	2.56	C6 ...H101	2.60
H61 ...C7	2.19	H61 ...C10	2.74
C7 ...H81	2.07	C7 ...C9	2.37
C7 ...H101	2.17	C7 ...H102	2.18
C7 ...H103	2.17	C8 ...H91	2.18
C8 ...C10	2.49	C8 ...H102	2.77
C8 ...H103	2.81	H81 ...C9	2.16
H81 ...C10	2.66	C11 ...C12	2.39
C11 ...H122	2.71	C11 ...H123	2.70
C13 ...H141	2.15	C13 ...C15	2.39
C13 ...C16	2.74	C13 ...C17	2.36
C13 ...H181	2.11	C14 ...H151	2.13
C14 ...C16	2.38	C14 ...C17	2.72
C14 ...C18	2.36	H141...C15	2.11
C15 ...H161	2.15	C15 ...C17	2.36
C15 ...C18	2.77	H151...C16	2.15
C16 ...H171	2.09	C16 ...C18	2.40

H161...C17 2.09 C17 ...H181 2.17

H171...C18 2.15